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# **Clinical and genetic risk factors in acute coronary syndromes**

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ACADEMIC DISSERTATION

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## List of original publications

This thesis is based on the following publications:

- I            Vaara S, Nieminen MS, Lokki ML, Perola M, Pussinen PJ, Allonen J, Parkkonen O, Sinisalo J. Cohort profile: The Corogene Study. *Int J Epidemiol.* 2012 Oct;41(5):1265-71.
  
- II            Vaara S, Tikkanen E, Parkkonen O, Lokki ML, Ripatti S, Perola M, Nieminen MS, Sinisalo J. Genetic risk scores predict recurrence of acute coronary syndrome. *Circ Cardiovasc Genet.* 2016 Apr;9(2):172-8.
  
- III            Salo PP, Vaara S, Kettunen J, Pirinen M, Sarin AP, Huikuri H, Karhunen PJ, Eskola M, Nikus K, Lokki ML, Ripatti S, Havulinna AS, Salomaa V, Palotie A, Nieminen MS, Sinisalo J, Perola M. Genetic variants on chromosome 1p13.3 are associated with non-ST elevation myocardial infarction and the expression of DRAM2 in the Finnish population. *PLoS One.* 2015 Oct 28;10(10):e0140576.
  
- IV            Vaara S, Salo P, Perola M, Parkkonen O, Lokki ML, Havulinna A, Salomaa V, Nieminen MS, Sinisalo J. A risk locus for non-ST-elevation myocardial infarction on chromosome 1p13.3 is also associated with peripheral artery disease in patients with acute coronary syndrome. (Submitted to *Scientific Reports*)

The publications are referred to in the text by their Roman numerals.

Additional information not published in these articles is included and cited as “S.V. et al., unpublished results”.

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## Most important abbreviations

ABI	ankle brachial index
ACS	acute coronary syndrome
AMI	acute myocardial infarction
ARIC	Atherosclerosis Risk in Communities
ASAT	aspartate aminotransferase
AUC	area under the receiver operating characteristics curve
BMI	body-mass index
CABG	coronary artery by-pass grafting
CAD	coronary artery disease
CCTA	computed coronary tomography angiogram
CI	confidence interval
CKMBm	creatine kinase-MB-mass
CRP	C-reactive protein
CVD	cardiovascular disease
DNA	deoxyribonucleic acid
DRAM2	damage-regulated autophagy modulator 2
ECG	electrocardiogram
FDR	false discovery rate
GFR	glomerular filtration rate
GGT	gamma-glutamyltransferase
GRACE	Global Registry of Acute Coronary Events
GRS	genetic risk score
GTex	Genotype-Tissue Expression project
GUSTO	Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries
GWAS	genome-wide association study
EMMAE	Evaluation of the Methods and Management of Acute Coronary Events
HbA1c	glycosylated hemoglobin A1c
HDL	high-density lipoprotein
HR	hazard ratio
IQR	interquartile range
LDL	low-density lipoprotein
LD	linkage disequilibrium
MAF	minor allele frequency
MDRD	Modification of Diet in Renal Disease
MI	myocardial infarction
MONICA	Multinational MONItoring of trends and determinants in Cardiovascular disease -Project
NSTEACS	non-ST-elevation acute coronary syndrome
NSTEMI	non-ST-elevation myocardial infarction
OR	odds ratio



PAD	peripheral artery disease
PCI	percutaneous coronary intervention
proBNP	Type B N-terminal natriuretic propeptide
PURSUIT	Platelet Glycoprotein IIb/IIIa in Unstable Angina: Receptor Suppression Using Integrilin Therapy
ROC	receiver operating characteristics
SD	standard deviation
SMC	smooth muscle cell
STEMI	ST-elevation myocardial infarction
SNP	single nucleotide polymorphism
SRI	Simple Risk Index
TACOS	Tampere Acute Coronary Syndrome Study
TIA	transient ischemic attack
TIMI	Thrombolysis in Myocardial Infarction Study Group
TnI	cardiac troponin I
TnT	cardiac troponin T
UAP	unstable angina pectoris

# Abstract

## *Aims*

This study aimed to investigate how various genetic variants affect acute coronary syndrome (ACS): its subtype and the prognosis. More precisely, its objectives were to describe the characteristics of a Finnish cohort of consecutive angiography patients and to evaluate the importance of clinical risk factors and genetic risk scores (GRS) in predicting the ACS prognosis. Additionally, genetic differences were sought between patients with non-ST-elevation myocardial infarction (NSTEMI) and ST-elevation myocardial infarction (STEMI). The newly discovered NSTEMI risk variant was studied for effects on prognosis, clinical risk factors and other manifestations of atherosclerosis.

## *Material and methods*

Studies I to IV are based on the Corogene cohort, consisting of 5 294 consecutive patients who underwent coronary angiography between 2006 and 2008. The patients with ACS or prior myocardial infarction (MI) were genotyped. Additionally, genotyped individuals from Tampere Acute Coronary Syndrome Study (TACOS) (Study III) and FINRISK cohorts (Studies III-IV) were included to further test the findings discovered of the Corogene Study.

Study I was an observational cohort study of all the 5 294 consecutive coronary angiography patients included in the Corogene Study. It described the risk factor profiles, disease stability and severity, and the revascularization strategy of all these patients. Study II elucidated the recurrence rate and the mortality from coronary artery disease (CAD) in the 2 090 ACS patients during a median 5.5-year follow-up, and clinical factors affecting their prognosis. The GRS of 47 previously known CAD risk variants was formed and tested for association with recurrent ACS and CAD mortality. Clinical risk factors were studied for association with the GRS47. Patients with MI were selected for Study III, a genome-wide association study (GWAS), to discover new genetic variants conferring risk specifically for NSTEMI or STEMI. The variants passing a pre-defined statistical significance threshold were chosen for replication in the TACOS and FINRISK cohorts. In Study IV, data of all the genotyped ACS patients served for analysis of clinical characteristics and comorbidities associated with the variant discovered in the GWAS of Study III, with special emphasis on the variant's effect on the prognosis and on incidence of peripheral artery disease (PAD).

## *Main results*

The most common reason for coronary angiogram in the Corogene Study during the study period was ACS (2 090, 39%). Almost one-fourth of the patients (1 207, 23%) had no signs of obstructive CAD in coronary angiography. About half of all patients (2 854, 54%) were finally revascularized.

Of the ACS patients, 263 (12.6%) had recurrent ACS, 455 (21.8%) died during the follow-up, and 259 (12.4%) had a CAD-related cause of death. The GRS47 was found associated with recurrent ACS, independent of clinical factors. When added to a risk-

prediction model of traditional risk factors and patient characteristics, however, the GRS47 did not improve the classification clinically significantly. Among ACS patients, the GRS47 correlated inversely with STEMI and with smoking. GWAS revealed genetic variants at a locus near 1p13.3, including rs656843, to be associated nominally with NSTEMI. Finally, rs656843 additionally showed association with PAD incidence in the ACS patients, but had no effect on recurrence of ACS or on survival during the follow-up. In the FINRISK cohort, which included healthier and younger individuals, no association could be demonstrated with PAD.

### ***Conclusions***

A GRS combined of CAD risk variants was associated with recurrent ACS in a Finnish ACS population. As it did not improve the accuracy of prediction by clinical factors, the GRS has thus not yet proven useful in clinical practice. Smoking, having an inverse association with the GRS, may outweigh the genetic predisposition to CAD.

The inverse correlation of GRS with STEMI and thus positive correlation with NSTEMI, the finding of a genetic variant linked nominally to NSTEMI, and the fact that patients with NSTEMI and STEMI present with different clinical risk-factor profiles suggest that these two ACS subtypes may have somewhat different etiologies. The genetic variant conferring the risk for NSTEMI was also associated with PAD in ACS patients. The heterogeneity in PAD phenotypes and genetics may explain why the association could not be shown in a general population sample but only in a selected cohort of ACS patients.

# 1. Introduction

Coronary artery disease (CAD), acute coronary syndromes (ACS), and other conditions related to atherosclerosis are leading causes of death in developed countries. Therefore, the search for risk factors has also been thorough. Known clinical risk factors for the different manifestations of cardiovascular disease (CVD), such as CAD, peripheral artery disease (PAD), ischemic stroke, and aneurysms of the aorta are overlapping, and the importance of one risk factor may vary across the disease subtypes. The most important and widely acknowledged risk factors are hypertension, dyslipidemia, diabetes mellitus, tobacco smoking, and genetics. While knowledge of the risk factors has increased, and both prevention and treatment of CAD and ACS have developed, CAD incidence and mortality have begun to decrease.

The gold standard in imaging CAD is invasive coronary angiography. Percutaneous coronary intervention (PCI) performed in the same session as coronary angiography is a cornerstone of ACS treatment. In stable CAD, in patients with severe symptoms, the angiography result guides the choice of revascularization strategy, and in patients with chest pain of unknown cause, the angiography may provide the diagnosis. In recent years, other methods for CAD imaging have also been rapidly evolving.

The incidence of CAD cannot be explained by environmental factors only; family history also matters. The genetics of CAD has been of great interest recently. During the last decade major findings have emerged with many CAD-related SNPs found. To date, more than 50 genetic variants have been linked to CAD. Of note, most of them do not act through any known clinical risk factor, but independently, or their mechanism of action remains unknown.<sup>159</sup> Thus, intensive research has focused on the question whether genetic risk variants, either as single variants or combined as genetic risk scores (GRS), can supplement clinical risk-prediction tools and help to predict an individual's risk for CAD and ACS more precisely.<sup>1,19,21,28,83,104,112,139,180,227,231,242</sup> Even though not used in clinical practice, GRSs have shown capability to predict CAD in healthy subjects. The role of GRSs in predicting recurrent ACS remains, however, unclear.

The ACS can be classified into ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI) and unstable angina pectoris (UAP). Patients with STEMI usually have fewer clinical risk factors, milder atherosclerosis observed in the coronary angiogram, and they are younger than are patients with NSTEMI.<sup>127</sup> Differences occur in the pathophysiology between the two conditions, and the acute management of these conditions differs substantially. Additionally, patients seem prone to either type of myocardial infarction (MI): in patients suffering from recurrent MI, the recurrent event is often of the same type as the first.<sup>186</sup> This raises the question whether differences also exist in the genetic background of these two conditions.

More risk variants are revealed for CAD than for other CVD subtypes, which can be explained by the broad spectrum of the disease phenotypes and a more heterogeneous genetic background. Some of the known genetic CAD risk variants also confer a risk for other manifestations of atherosclerosis; they are thus pleiotropic. Consequently, each new CAD/MI risk variant should be evaluated in relation to other CVD manifestations.<sup>107</sup>

This study aimed to investigate the effects of genetic variants on ACS: ACS subtype and prognosis. Additionally, this study describes the characteristics and findings of a cohort of consecutive patients who underwent coronary angiography, and evaluates the discovered NSTEMI risk variant in terms of pleiotropy.

## 2. Review of the literature

### 2.1. Epidemiology of CAD and ACS

CAD and ACS are leading causes of death in the developed countries, and CAD is the most frequent cause of death worldwide. Every year over seven million people die of CAD, which is 12.8% of all deaths worldwide.<sup>247</sup> In Europe, CAD causes 20% of all deaths: 1.8 million annually.<sup>158</sup>

In Finland, for CAD and ACS altogether, hospital admissions annually number more than 60 000, of which 13 000 are for ACS. The number of patients suffering from ACS was decreasing steadily during 1990s, it peaked in 2000-2003, and has then trended downward again.<sup>219</sup> CAD mortality in Finland has also decreased since the 1970s. The most important reasons for the decline are decreases in risk factors, such as dyslipidemia, smoking, and hypertension, in addition to development in treatment strategies.<sup>236</sup>

The main reason for the substantial decrease in CAD mortality, at least in Finland and Norway, during the past decades is considered to be a decreased prevalence of dyslipidemia.<sup>110,128,236</sup> However, according to the FINRISK Study, after 2007 the cholesterol level has risen again, most likely stemming from the public debate around the dietary lipids and the benefits of statins.<sup>236</sup>

Whereas in older generations men smoked more often than women, in younger generations this gender gap seems to be narrowing. While smoking prevalence in younger women is increasing in some regions of the world, it is declining in men.<sup>109</sup> This change in smoking pattern will most likely cause a substantial increase in MI rates in women.<sup>218</sup> In Finland, however, tobacco smoking in women, after peaking in 2007, has dropped. Still, when compared to about 10% in the 1970s, the rate of tobacco smoking had doubled by 2012. In contrast, the prevalence of smoking in men has declined from about 50% in 1971 to 27% in 2012.<sup>94</sup>

The overall prevalence of hypertension is estimated to be around 30 to 45%.<sup>126</sup> With antihypertensive drug-use or blood pressure over 140/90 mmHg as the criterion, as many as half of all Finnish men and two-fifths of women over age 30 are considered hypertensive.<sup>103</sup> The mean blood pressure in Finns decreased from 1970 to 2000 in all age groups,<sup>95</sup> but started to rise again in 2010.<sup>111</sup>

Incidence of STEMI has decreased over the last decade, whereas incidence of NSTEMI has increased, possibly resulting from changes in the prevalence of and improved treatment for the main risk factors. Short-term mortality from STEMI is higher than from NSTEMI, but during one to two years of follow-up, the difference in mortality rates evens out.<sup>135</sup> Patients with NSTEMI are older and have more co-morbidities than do patients with STEMI.<sup>127</sup> The known factors related to STEMI mortality include age, Killip class,<sup>100</sup> time-delay to treatment, mode of treatment, history of prior MI, diabetes mellitus, renal insufficiency, severity of CAD disease in angiography, ejection fraction, and treatment.<sup>127</sup>

## 2.2. Classification and diagnosis of stable CAD and ACS

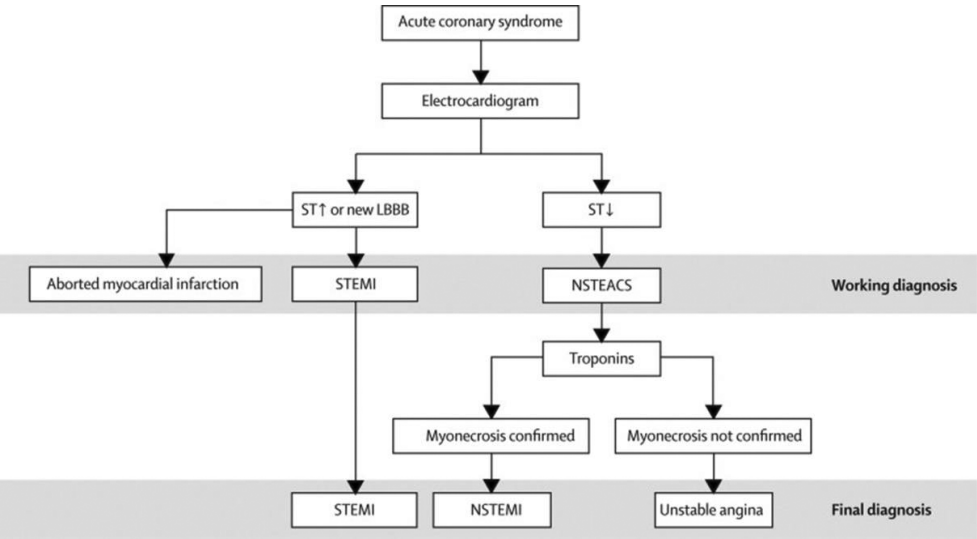
In CAD, oxygen supply to the myocardial cells is diminished due to obstruction of the coronary arteries. The resultant imbalance in oxygen supply and demand leads to clinical symptoms. In stable CAD, this imbalance is reversible, and is usually caused by a combination of narrowed coronary arteries and increased oxygen demand in situations like exercise. After the exercise ceases, oxygen demand returns to normal, and the symptoms ease.<sup>145</sup> In ACS, including MI and UAP, the imbalance is due to the sudden obstruction of a coronary artery, and in MI this imbalance leads to myocardial damage due to prolonged ischemia.<sup>184,207</sup>

Ischemia causes typical symptoms: acute chest pain, possibly radiating to the lower jaw and left arm, epigastric discomfort, dyspnea, and fatigue.<sup>145</sup> Whereas in stable CAD these symptoms are reversible, in ACS the symptoms are worse and typically last more than 20 minutes. The ischemia may, however, also be silent or atypical, including diffuse discomfort, nausea, palpitations, or cardiac arrest. Women, the elderly, patients with diabetes, and critically ill patients are particularly prone to these atypical manifestations.<sup>226</sup>

Diagnosis of both stable CAD and ACS is based on patient history, symptoms typical for ischemia, risk-profiling, physical examination and an electrocardiogram (ECG).<sup>145,184,207</sup> Myocardial ischemia causes injury currents: alterations in the repolarization and depolarization of cardiomyocytes detectable as changes in the ECG.<sup>43,191</sup> The diagnostics is typically supplemented with exercise ECG testing in stable CAD, because ECG changes are reversible and may usually be detectable only during exercise.<sup>55</sup> In ACS, measurement of cardiac biomarkers is crucial for diagnostics.

The Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) Project, coordinated by World Health Organization (WHO), defined uniform criteria for MI in the 1980s. These criteria for a definite MI included typical chest pain symptoms and either ECG susceptible for ischemia, including the development of Q-waves, or cardiac biomarker value double the value of the normal upper limit.<sup>248,249</sup> At that time, the cardiac biomarkers used were aspartate aminotransferase (ASAT), lactate dehydrogenase, and creatinine kinase (CK) and its MB isoenzyme. These biomarkers, however, may also rise for many reasons other than MI, and to include only definite MIs, the cut-off value was defined as considerably high. When the sensitivity and specificity of biomarkers developed, and the ability to identify MI improved, the criteria for MI were redefined in 2000.<sup>6</sup> In the 2000 definition, the ACS were classified at presentation by the presence of ST-elevation, and further by evolving ECG changes confirming the myocardial necrosis and release of cardiac biomarkers to UAP, non-Q-wave MI and Q-wave MI. In 2007, the definition was updated and supplemented with a classification of the underlying reasons for MI.<sup>226</sup> The most recent update of the definition was made in 2012, with a revised definition of myocardial necrosis and reduced emphasis on biomarkers other than cardiac troponin.<sup>225</sup> According to this latest definition of MI, the crucial criterion for MI is detection of a rise/fall in cardiac biomarkers with at least one value above the 99<sup>th</sup> percentile upper reference limit, accompanied by at least one of the following criteria: symptoms of ischemia, new ST-segment/T-wave changes or left bundle branch block, development of Q-waves, new loss of viable myocardium or regional

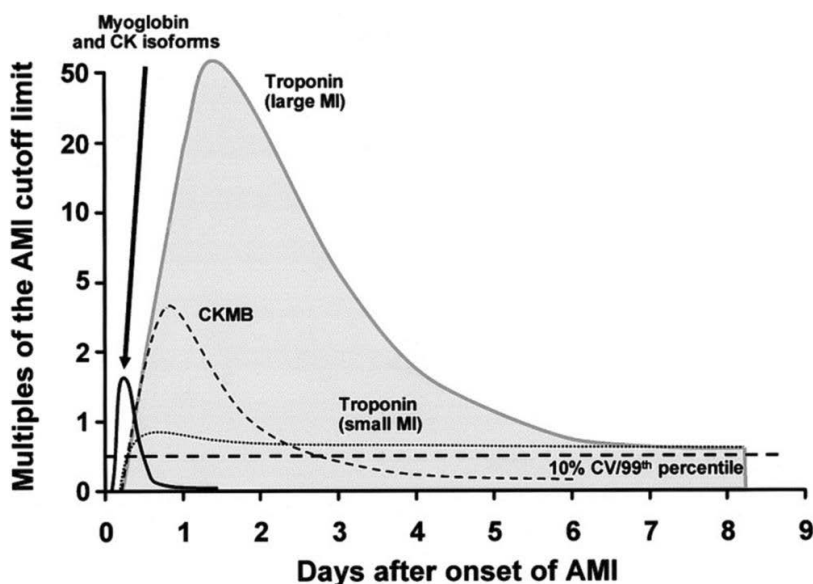
motion abnormality, or identification of intracoronary thrombus by angiography or autopsy.<sup>225</sup>



**Figure 1** *Classification of acute coronary syndromes. STEMI indicates ST-elevation myocardial infarction; NSTEACS, non-ST-elevation acute coronary syndrome; NSTEMI, non-ST-elevation myocardial infarction; LBBB, left bundle branch block. Reprinted from The Lancet, Volume 372, Issue 9638, 16–22 August 2008, White et al. Acute myocardial infarction. Pages 570–584.<sup>90</sup> Copyright (2008), with permission from Elsevier.<sup>246</sup>*

Figure 1 presents the classification of ACS. At presentation, the ACS can be classified into three categories: STEMI, non-ST-elevation ACS (NSTEACS) and aborted MI. This categorization is essential due to differing treatment approaches and protocols between the conditions.<sup>184</sup> STEMI generally reflects a total coronary occlusion. The ECG criteria for STEMI are persistent ST-segment elevation in at least two contiguous leads or a new left bundle branch block.<sup>207</sup> In NSTEACS, although transient ST-segment elevation, ST-segment depression, T-wave inversions or flat T-waves may be present, no persistent ST-elevation is detectable. The ECG can also be normal.<sup>184</sup> NSTEACS is further classified into NSTEMI if cardiac biomarkers, indicating myocardial damage, are released, and is called unstable angina UAP if biomarker release is undetectable.





**Figure 2** Time course of the appearance of various markers in the blood after acute myocardial infarction (AMI). Shown are the time concentrations/activity curves for myoglobin and creatine kinase (CK) isoforms, troponin after large and small infarctions, and CK-MB. Note that with cardiac troponin some patients have a second peak in addition. CV = coefficient of variation. Reprinted from *Journal of the American College of Cardiology*, Volume 48, Issue 1, 4 July 2006, Jaffe et al. *Biomarkers in Acute Cardiac Disease: The Present and the Future*. Pages 1–11.<sup>90</sup> Copyright (2006), with permission from Elsevier.

The release of cardiac biomarkers into the blood flow indicates myocardial damage due to prolonged ischemia. Today, the most often used cardiac biomarkers are cardiac troponin T and I (TnT, TnI), and creatine kinase-MB-mass (CKMBm). These biomarkers stay in the blood flow different times (Figure 2). After the introduction of high-sensitivity cardiac troponin assays, the number of patients with UAP has dropped and the number of patients with NSTEMI has risen accordingly. This change arises from the fact that many patients earlier considered to have UAP due to negative troponin are now diagnosed with NSTEMI, because today even a minor release of troponin is detectable.<sup>23</sup> Patients with UAP do not suffer from myocardial necrosis and thus benefit less than do NSTEMI patients from early invasive strategy and intensive antiplatelet therapy.<sup>23,152,174</sup> In Helsinki University Hospital, the use of high-sensitive-troponin began in 2011.

Depending on the causes of ischemia and prognostic differences, MI can be classified into five types (Table 1).<sup>225,226,246</sup> Type 1 is spontaneous MI. It is related to the rupture, ulceration, erosion or fissuring of the atherosclerotic plaque, leading to thrombosis, rapid occlusion of a coronary artery and thus to myocardial ischemia and necrosis. These patients may have severe CAD, but angiography shows that some patients have a non-obstructive CAD or no CAD at all.<sup>24,177,183</sup> In type 2 MI, the ischemia is secondary and caused by an imbalance in oxygen supply and demand on the myocardium by conditions

other than CAD, such as endothelial dysfunction, coronary artery spasm, embolisms, arrhythmias, anemia, respiratory failure, or hypotension or hypertension.<sup>225</sup> Type 3 MI is cardiac death with preceding symptoms and ECG typical of ischemia, before cardiac biomarkers were available. Type 4 and 5 MI are associated with preceding revascularization procedures, i.e. PCI and coronary artery by-pass grafting (CABG).<sup>225</sup>

**Table 1.**        *Classification of myocardial infarction by the underlying reason.*

<b>Type</b>	<b>Description</b>
1	Spontaneous myocardial infarction
2	Infarction secondary to other conditions causing imbalance in oxygen supply and demand
3	Cardiac death with preceding symptoms and ECG typical for ischemia
4a, 4b, 4c	Infarction related to a) percutaneous coronary intervention, b) stent thrombosis, or c) restenosis
5	Infarction related to coronary artery by-pass grafting

*ECG indicates electrocardiogram. Data obtained from Thygesen et al.* <sup>225</sup>

## 2.3. Pathophysiology

### 2.3.1. Formation and composition of an atherosclerotic plaque

CAD is a multifactorial disease, developing in combination with hyperlipidemia, especially involving increased serum concentration of low-density lipoproteins (LDL), and other risk factors such as smoking, hypertension, diabetes mellitus, male sex, and family history. In a typical patient with advanced CAD numerous plaques of different phases are present at different sites in the coronary arterial tree.<sup>16</sup> To induce the disease requires higher LDL levels than to sustain progression once lesions have formed.<sup>16,68</sup>

The process of plaque formation begins with adaptive intimal thickening in response to mechanical stress affecting especially the vessel's inner curvatures and branch points. This process begins spontaneously in early childhood. Mechanical stress leads to changes in endothelial metabolism and gene expression.<sup>244</sup> Pathological intimal thickening is already observable in the coronaries at age 20 to 30. Over time, the arterial wall remodels, and plaque develops, which changes the local flow patterns.<sup>16</sup>

LDLs accumulate in the thickened intima, aggregate, and oxidize. The modified LDLs stimulate the immune process and induce, for example, differentiation of monocytes into several phenotypes of macrophages and dendritic cells.<sup>118</sup> In turn, the immune cells further promote the development of lesions and inflammation.<sup>16</sup> Macrophages and dendritic cells, after their up-take of LDL, become foam cells and lipid deposits. Foam cells layer within the proteoglycan of the intima and form fatty streaks, also called as intimal xanthomas, which can still be reversible.<sup>16</sup> However, if lipid-rich material still keeps accumulating in the intima, fatty streaks further develop into progressive atherosclerotic lesions.<sup>205</sup>

When the macrophage invasion and the resultant growth of lipid pools form a necrotic core, the plaque becomes a fibroatheroma. The necrotic core contains apoptotic remnants of cells. In a late necrotic core, no matrix is left, and the changes are irreversible.<sup>101</sup> Men and women develop the same amount of fatty streaks early in life, but by the age of 30, men have developed more progressive atherosclerotic lesions.<sup>134</sup> Leaky neovessels grow into the base of atherosclerotic lesions. These neovessels are fragile and have few supporting cells, and thus they allow the intraplaque bleeding common in fibroatheromas. Neovascularization and intraplaque bleeding expand the necrotic core and promote inflammation.<sup>203</sup> Extravasation can also happen through a ruptured fibrous cap that remains between the necrotic core and the vessel lumen.

Progressive atherosclerotic lesions can be calcified to varying extents. Calcification increases with age and can constitute most of the plaque volume. The necrotic core can completely calcify. Some plaques can even consist of fibrous and calcified tissue only, without any necrotic core or extracellular lipids.<sup>204</sup>

At the time of atherosclerotic plaque development, the local vessel segment is also remodeled. This remodeling can be expansive, i.e. the lumen area is usually not reduced until plaques become very large. The remodeling can also be constrictive, which leads to shrinkage of the vessel segment and the lumen. Expansive remodeling is present in fibroatheromas and constrictive remodeling in more fibrous lesions. The mode and extent

of remodeling is crucial in determining the severity of the stenosis. Due to remodeling, it is impossible to exclude the presence of atherosclerotic plaques by invasive angiography.<sup>27</sup>

### **2.3.2. Pathophysiology of ACS**

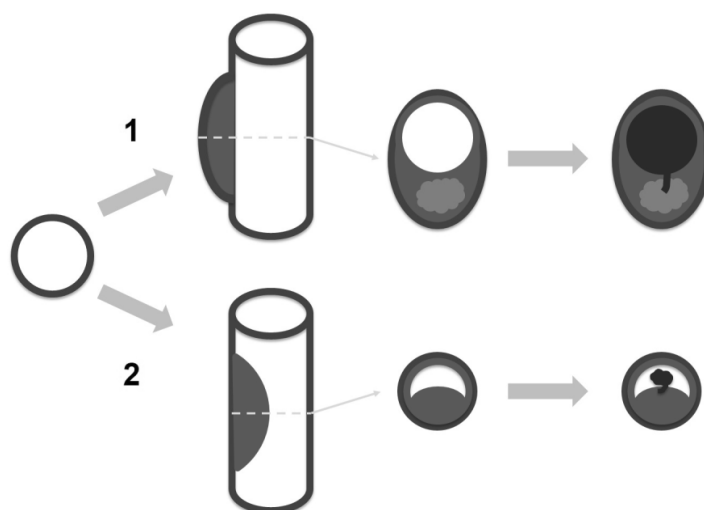
Sudden obstruction of a coronary artery results in impaired blood flow to those parts of the myocardium that the vessel segment supplies. This leads to an inadequate supply of oxygen and results in ischemia. The ischemia causes the typical symptoms and ECG changes, whereas the plaque disruption itself is symptomless.<sup>16</sup> The ischemia may lead to myocardial necrosis, causing the release of cardiac biomarkers such as troponin. Atherosclerosis alone can cause symptoms of stable angina pectoris, but ACS almost always results from a luminal thrombus or a plaque hemorrhage.<sup>37,176,228</sup>

The cause of coronary thrombosis is usually a plaque rupture (Figure 3). Rupture of a fibrous cap exposes the thrombogenic core to the blood stream. Plaque rupture plays a key role in thrombi that cause MI both in patients who survive and in those who die.<sup>117</sup> Smoking is known to predispose every type of coronary plaques to thrombosis. Plaque ruptures occur more in patients with STEMI than with NSTEMI.<sup>117</sup>

Ruptures are likely in plaques with a thin fibrous cap and a large necrotic core infiltrated with macrophages.<sup>10</sup> In eccentric plaques, the weakest spots are the cap margins and shoulder region. Infiltrating macrophages degrade the cap matrix, and smooth muscle cells (SMC) leave the cap, which leads to thinning of the fibrous cap.<sup>117</sup> Intraplaque macrophages overproduce matrix-metalloproteinases, which have interstitial collagenase activity and thus catalyze the attack on fibrillar collagen.<sup>200</sup> It remains unclear how long the fibrous cap thinning takes, but this is assumed to be a slow process of years or decades. Rupturing of a fibrous cap can happen spontaneously, but physical or mental stress or activation of the sympathetic nervous system can be the trigger for rupture.<sup>16</sup>

Other features associated with plaque vulnerability and rupture are spotty calcification, adventitial and perivascular inflammation, neovascularization from the vasa vasorum and plaque hemorrhage, and expansive remodeling. Many angiographic studies indicate that lesions causing ACS actually are often non-occlusive.<sup>117,228</sup> Studies by computed coronary tomography angiogram (CCTA) and intraluminal ultrasound have shown that plaques associated with ACS include outward expansion of the artery wall and only some calcification, and they lie proximal to the sites of maximal stenosis.<sup>150,196</sup>

Erosion of plaques can lead to thrombosis. Such eroded plaques are more often calcified, less inflamed, and are more often associated with constrictive remodeling than are the ruptured plaques. Plaque erosion is a weaker thrombogenic stimulus than is a rupture. Plaque erosion occurs more frequently in women than in men, with hypertriglyceridemia in particular being linked to erosion.<sup>117</sup>



**Figure 3** *The pathogenesis of acute coronary syndrome. Ruptures (1) and erosions (2) of the atherosclerotic plaques lead to acute coronary syndromes. Ruptures are common in plaques with a thin fibrous cap and a large necrotic core (1). Because of expansive remodeling, these kinds of plaques are often non-occlusive. Rupture of the plaque leads to thrombosis and sudden total occlusion of the coronary artery. Plaques associated with erosion are more often calcified and associated with constrictive remodeling (2). Erosion is a weaker thrombogenic stimulus than rupture.<sup>16</sup>*

In patients suffering from CAD, many healed ruptures are often present in the coronary artery tree. The number of ruptures correlates with the severity of stenosis. Silent erosion, rupturing, and healing are important for plaque growth and further stenosis. Negative remodeling is often related to severe stenosis.<sup>16</sup>

Local inflammation seems to play a key role in plaque disruption, and the systemic inflammatory response to MI can worsen the inflammation in all of the plaques, as well. This explains why recurrent thrombotic events happen soon after ACS and involve lesions not culprits in the first event. Furthermore, this explains why immediate revascularization that limits myocardial injury and consequently also systemic inflammation, reduces recurrent events more efficiently than does revascularization occurring later after ACS.<sup>117</sup> Statin treatment also has prevented the first and subsequent ACS in many patient categories, even though it has a low impact upon actual degree of stenosis.<sup>156</sup> Both statin treatment and a lipid-lowering diet reduce the content of lipids and inflammatory cells and increase the fibrous nature of plaque, thus most likely stabilizing plaques.<sup>4,146,214</sup>

Small pre-existing but not working connections between the main coronary arteries, i.e. collaterals, may be present, although in coronary angiography they may be invisible. They develop slowly, and when a pressure gradient develops as the native coronary artery occludes, they open.<sup>194</sup> Both chronic ischemia and slow narrowing of the coronary arteries stimulate growth in the caliber of the collaterals. Collateral function is positively associated with CAD severity.<sup>199</sup> In chronic total obstruction of a coronary artery, the blood flow through the collaterals prevents severe ischemia and myocardial

necrosis.<sup>33,199,245</sup> Collateral development may be one of the reasons why older patients with excessive atherosclerosis suffer relatively more often from NSTEMI than from STEMI.

## 2.4. Clinical risk factors of atherosclerosis and ACS

Because CAD has proven a leading cause of death and demanded considerable health care resources for decades, the search for risk factors for the condition has also been extensive. The Framingham Study, the landmark study of the CAD risk factors starting back in 1948, carefully monitored more than 5 000 individuals and identified physical inactivity, high blood pressure, diabetes, blood-lipid profile, and cigarette smoking as major CAD risk factors.<sup>125</sup> In the 1980s, The Framingham Study and other studies had already suggested more than 200 risk factors.<sup>80</sup> CAD prevalence increases with age. Male gender is a well-known risk factor for CAD and MI. Although the percentage of total deaths from CAD between genders is approximately the same, premature death before age 65 and 75 is more common in males.<sup>158</sup>

A crucial risk factor for CAD and MI is dyslipidemia. LDL is a key contributor to CAD development. If LDL level is low, clinically relevant CAD cannot develop, regardless of other risk factors.<sup>208</sup> The risk factors each act differently in atherosclerosis development and thus link slightly differently to CVD presentations. For example, smoking predisposes more to MI than to stable CAD,<sup>22</sup> hypertension especially to stroke,<sup>151</sup> and smoking and diabetes mellitus to PAD.<sup>54</sup> Moreover, LDL level elevates the risk for CAD more than for PAD and stroke.<sup>54,253</sup> For example, in patients with familial hypercholesterolemia, the risk for fatal stroke does not seem to be as increased as the risk for CAD.<sup>84</sup>

High serum levels of LDL raise LDL accumulation in the arterial intima. In addition, LDL is linked to the inflammation associated with vulnerable plaques. Regardless of the method by which LDL is lowered, the risk for MI is reduced.<sup>26,118</sup> In contrast to LDL, HDL removes lipid particles from the arterial wall. It also acts as an antioxidant and has anti-inflammatory properties. However, the interventions to raise HDL level have proven of no clinical benefit.<sup>118,240</sup> In addition to LDL and HDL cholesterol, triglyceride level is also associated with CAD, but it has not been confirmed as a causal risk factor. The level of triglycerides is highly associated with other CAD risk factors such as diabetes mellitus, but when adjusted for other risk factors, triglycerides have not been associated with CAD.<sup>118</sup>

Tobacco smoking is associated with an increased risk for acute MI, and is even one of the largest contributors to MI worldwide. Smoking promotes atherosclerosis by causing endothelial dysfunction, oxidation of proatherogenic lipids, decrease in HDL and induction of inflammation.<sup>142</sup> Smoking is additionally linked to coronary thrombosis.<sup>12,26</sup> When compared to never-smokers, smokers have a three-fold risk for MI.<sup>218</sup> This risk is greater in the young than in the old, and correlates with the amount of tobacco used, but even low tobacco consumption associates with an elevated MI risk. After smoking cessation, the risk starts to decrease over time, but remains higher than in non-smokers.<sup>218</sup>

Women tend to be even more sensitive to tobacco. Smoking women have an up to 25% higher risk for CAD than do smoking men.<sup>85</sup>

Hypertension not only enhances the CAD development, but also adds to the workload of the heart, thus causing an increased oxygen demand in cardiomyocytes. Over the long run, hypertension can cause heart failure.<sup>46</sup> Hypertension eventually reduces arterial elasticity and promotes atherosclerotic plaque formation. A link between inflammation, hypertension, and atherosclerosis is angiotensin-II, which, besides its action as a vasoconstrictor, is also an initiator of intimal inflammation. It increases expression of proinflammatory cytokines on endothelial cells.<sup>119</sup> Along with elevated blood pressure, CAD risk increases without any threshold value. It has been estimated that each blood-pressure increase with 20/10 mmHg at least doubles CAD mortality.<sup>116</sup> Systolic blood pressure and pulse pressure increase slowly with age.<sup>57</sup>

Diabetes mellitus is a major contributor to CAD development. It includes a range of conditions, the two most common being juvenile-onset diabetes (type 1) and adult-onset diabetes (type 2). Most diabetics are of type 2, which often appears together with other CAD risk factors as well. Type 2 diabetes is characterized by impaired glucose tolerance, which results from development of insulin resistance. The glycosylated hemoglobin percentage, reflecting high blood-glucose values, has been associated with subclinical CAD independently of other risk factors even in non-diabetic patients,<sup>32</sup> and it slightly sharpens CAD-risk prediction.<sup>42</sup> Additionally, diabetes promotes oxidative stress, contributes to the production of proinflammatory cytokines, and enhances other inflammatory pathways in endothelial cells.<sup>14</sup> Thus, inflammation serves as a link between diabetes and atherosclerosis.<sup>119</sup>

Obesity not only elevates risk for CAD and ACS but also the risk for diabetes, dyslipidemia, and overall atherosclerosis. Moreover, adipose tissue promotes inflammation by synthesizing cytokines.<sup>119</sup> Obesity is a major problem all over the world, especially in developing countries. For approximating ACS risk, waist-to-hip and waist-to-height ratios are considered more useful than body-mass index (BMI).<sup>195,261</sup> In metabolic syndrome, the main risk factors, except for smoking, are all linked. Insulin resistance, hyperglycemia, hyperleptinemia, hypertension, and dyslipidemia, all present in metabolic syndrome, enhance atherosclerosis by differing mechanisms.<sup>209</sup>

Inflammation links the main risk factors and marks atherosclerosis development. Inflammatory cells in atherosclerotic lesions secrete proinflammatory cytokines, which induce hepatic C-reactive protein (CRP) production. In some studies, elevated CRP has predicted cardiovascular risk even better than does LDL level. CRP also correlates with CAD severity.<sup>178,179</sup>

Risk factors for CAD probably vary between populations, with lipids not being associated with CAD in south Asians, in the Chinese and in the Japanese, where high blood pressure may, in turn, play a larger role.<sup>263</sup> Moreover, prevalence of these risk factors varies among populations. The INTERHEART Study, a large multicenter cohort purposed to study CAD risk factors in different populations, evaluated nine easily measured, potentially modifiable risk factors to account for as much as 90% of the risk for the first MI. This effect is consistent in men and women, and across geographic regions and ethnic groups.<sup>262</sup> The INTERHEART study defines tobacco smoking and an abnormal blood lipid profile as the most important risk factors, other factors accounting for the risk

being diabetes, hypertension, abdominal obesity, psychosocial factors, and lifestyle factors including low fruit and vegetable intake, physical inactivity and alcohol use.

Women suffering from any type of ACS are older, and more likely to be hypertensive and diabetic than are their male counterparts. Instead, men with ACS are more likely to be smokers.<sup>5,7,75</sup> Several studies of coronary angiography patients demonstrate that men tend to have angiographically more severe CAD than do women.<sup>17,41,105</sup> Age and presence of traditional risk factors correlates positively with the number of diseased coronary arteries, but these have no effect before age 65.<sup>241</sup>

## **2.5. Treatment of stable CAD and ACS**

Treatment of stable CAD includes treating all diagnosed risk factors and preventing any that the patient does not yet have by means of lifestyle changes and correctly chosen medication. The aim of the treatment is to improve quality of life by easing symptoms, to prevent ACS, and to improve prognosis. If the patient suffers from difficult symptoms regardless of adequate medical therapy, revascularization by PCI or CABG can be considered.<sup>145</sup> Statin therapy improves the prognosis of CAD patients, and should therefore be prescribed for every CAD patient regardless of LDL level, in the absence of contraindications.<sup>86,157,165</sup> The goal of the lipid treatment is an LDL level under 1.8 mmol/L. Systolic blood pressure should be lowered to 135 mmHg and diastolic to 85 mmHg. The glycosylated hemoglobin A1c (HbA1c) should be lowered to under 53 mmol/mol. These goals should be taken into consideration while monitoring a patient with stable CAD and in choosing the combination of medications, but each patient's individual characteristics also matter.<sup>145</sup>

In ACS patients, the classification to either STEMI or NSTEMI guides the choice of acute treatment.<sup>184,207</sup> With the exception of antiplatelet treatment, management after the ACS is similar to the management of patients with stable CAD. Whereas in STEMI patients, an immediate revascularization by medical thrombolysis or PCI is crucial for survival, in NSTEMI patients the primary therapy is medical, with coronary angiography conducted within a few days after disease onset.<sup>11,184</sup> When compared to delayed revascularization strategy, revascularization conducted within 12 hours after symptom onset has been associated with a higher survival rate and reduction in development of large myocardial damage at 30 days.<sup>155</sup> However, another study showed no difference between these same groups in mortality, MI, or urgent revascularization at 30 days.<sup>144</sup>

In addition to the revascularization, intensive antithrombotic therapy is vital to treat ACS effectively, including both antiplatelet agents and anticoagulants.<sup>18,138</sup> Standard treatment in Finland includes acetylsalicylic acid combined with clopidogrel, prasugrel or ticagrelor as antiplatelet medication, and low-molecular-weight heparin.<sup>210,211</sup>



## 2.6. Genetics of CAD

Besides the traditional risk factors of atherosclerosis, genetics also plays a critical part in CAD development. Family history was already identified decades ago as an independent risk factor. CAD is a complex disease and heritable in a multifactorial manner, meaning the contribution of several genes together with environmental factors.<sup>181</sup> Genetic factors account for up to 30 to 60% of the variation in the CAD risk.<sup>121,130</sup> Even though the genetic risk for each individual is predefined, a healthy lifestyle may still prevent actual onset of the disease.

Genome-wide association studies (GWAS) have identified more than 50 loci that predispose to the development of CAD or MI.<sup>9,34,39,40,50,66,71,74,87,96,123,136,159,175,190,198,234,243,257</sup> GWAS compare the allele frequency of single nucleotide polymorphisms (SNPs) across the genome between cases and controls in study samples including thousands of individuals.<sup>197</sup> GWAS preferentially cover common SNPs with minor allele frequency (MAF) greater than 5 to 15% to achieve high statistical power for detecting associations. Consequently, most of the identified CAD risk alleles are quite common. The increasing number of identified risk loci and their high frequency in population means that most individuals are likely to carry several CAD risk variants.<sup>98</sup> The genetic risk for CAD is additive, and each risk allele contributes to the risk for a small proportion.<sup>98</sup>

Instead of being causal, the risk SNPs mark a linkage disequilibrium (LD) block, a particular region on a chromosome, in which the actual causal variant is located.<sup>98</sup> The majority of the risk SNPs locate in noncoding sequences. They exert their effect through mediation of nearby protein-coding sequences in the same chromosome and possibly also in other chromosomes.<sup>181</sup> Therefore, the discovery of a risk SNP leads to a search for the actual causal variant and the pathway behind the elevated risk. Some of the risk SNPs are suggested to affect expression of the nearby genes in a quantitative way, thus contributing to CAD risk.<sup>34</sup> Not all SNPs with the strongest effect are located near protein-coding regions. Since only a minority of the risk SNPs revealed seems to act via one of the traditional risk factors, for most of the risk SNPs, the mechanism remains unknown.<sup>181</sup> Further, in regions with a link discovered between a risk SNP and a specific gene, the majority of these genes have not been linked to CAD pathogenesis before. Knowledge of the mechanisms behind risk-allele associations is still incomplete.<sup>98</sup>

CAD genetics is suspected to be, at least in part, population-specific. Most of the GWAS for CAD have been performed in populations of European ancestry. However, a GWAS discovered loci related to CAD in Han Chinese.<sup>123</sup> These loci have not been linked to CAD in European populations, but they are associated with hypertension. A Japanese GWAS also found novel susceptibility loci for CAD.<sup>74</sup>

### 2.6.1. Revealed genetic risk variants for CAD

New risk variants for CAD/MI are emerging increasingly rapidly. The first CAD risk loci were discovered in GWAS performed in 2007. Since then, cohort sizes have increased, and the imputation methods have improved. The largest international cohorts this far,

including the CARDIoGRAMplusC4D Consortium, have discovered or replicated altogether more than 50 CAD risk variants (Table 2). Moreover, some variants show association with CAD only in specified populations, and some of the variants are awaiting replication. In addition to the genome-wide significant risk variants, dozens of suggestive variants strongly, but not genome-wide significantly, have been presented.<sup>40,159</sup>

The first and best-known locus found to be associated with CAD is 9p21, which was found simultaneously but independently by an American<sup>136</sup> and an Icelandic<sup>71</sup> research group. This common locus acts independently of known risk factors. The increased relative risk for CAD was 25% for heterozygotes and 50% for homozygotes.<sup>181</sup> The mechanism behind the risk is unknown, but strong evidence indicates that it is proatherogenic and acts at the vessel wall.<sup>76,92</sup> The 9p21 variant associates only with CAD, not with MI.<sup>81</sup> This variant is also associated with progression of CAD judged by the number of diseased coronary arteries.<sup>28</sup> In another study, this locus, however, was unrelated to the incidence of MI and mortality.<sup>81</sup>

Besides CAD, few data exist regarding the genetics associating directly with ACS or specifically with either STEMI or NSTEMI.<sup>182</sup> However, the variants affecting risk for ACS differ from those affecting atherosclerosis development; the former may instead contribute to thrombosis, plaque rupture, or release of tissue-coagulation factors.<sup>181</sup> Thus far, only the *ABO* blood group locus at 9q34.2 has shown an association with MI.<sup>175,189</sup> In a 20-year follow-up study, the A and B risk variants were associated with a 10% increased frequency of MI, and a combination of A and B elevated this risk to 20%.<sup>69</sup>

A locus at 1p13.3 is known to associate with diminished risk for both CAD and MI, acting in part through lower LDL levels.<sup>96,153,251</sup> In one study, this locus was associated with reduced risk of readmission for NSTEMI and a lower CVD readmission rate, independent of established predictors of increased risk.<sup>49</sup>

Chromosome 1q41 has been associated with CAD and MI in multiple GWAS. It elevates CAD risk by 14%.<sup>50,96,189,190</sup> In a study of three different cohorts, an independent association existed between the variant at 1q41 and survival/readmission. When compared with CC carriers, significantly fewer healthy participants carrying AC/CC experienced an NSTEMI throughout their follow-up.<sup>49</sup>

Risk factors are also under genetic regulation. Already more than 150 genetic risk variants are known to associate with blood lipid levels.<sup>220,252</sup> An accumulation of genetic risk variants can make patients unresponsive to treatment of their blood lipid levels. Additionally, multiple risk variants associated with hypertension and diabetes mellitus have emerged.<sup>48,147,193</sup>

**Table 2.** *Coronary artery disease and myocardial infarction risk variants and their use in risk prediction models.*

	Genes involved	Year	Original reference(s)	Function and possible relevance to CAD	Lead SNP and proxy SNPs	Included in risk scores	Minor/major allele
<b>1p13</b>	SORT1	2007	Samani <sup>190</sup>	LDL cholesterol <sup>181</sup>	rs599839	Tragante, <sup>231</sup> Krarup, <sup>104</sup> Bjornsson, <sup>19</sup> Bolton <sup>21</sup>	A/G
<b>1q21</b>					rs646776	Labos, <sup>112</sup> Mega, <sup>139</sup> Hughes, <sup>83</sup> Tikkanen, <sup>227</sup> Ripatti <sup>180</sup>	T/C
<b>1p32</b>	IL6R	2013	Deloukas <sup>40</sup>	Immune response <sup>137</sup>	rs4845625	Krarup, <sup>104</sup> Bjornsson <sup>19</sup>	T/C
	PPAP2B	2011	Schunkert <sup>198</sup>	Regulation of cell-cell interactions <sup>137</sup>	rs17114036	Labos, <sup>112</sup> Tragante, <sup>231</sup> Krarup, <sup>104</sup> Mega, <sup>139</sup> Bjornsson, <sup>19</sup> Tikkanen, <sup>227</sup> Bolton <sup>21</sup>	A/G
<b>1q41</b>	MIA3	2007	Samani <sup>190</sup>	Collagen secretion <sup>137</sup>	rs17465637	Labos, <sup>112</sup> Tragante, <sup>231</sup> Mega, <sup>139</sup> Bjornsson, <sup>19</sup> Tikkanen, <sup>227</sup> Ripatti, <sup>180</sup> Bolton <sup>21</sup>	C/A
					rs17464857	Krarup <sup>104</sup>	C/T
					rs17011666	Bolton <sup>21</sup>	G/A
					rs2133189		T/C
					rs3008621	Hughes <sup>83</sup>	A/G
<b>1p32</b>	PCSK9-BSND	2009	Kathiresan <sup>96</sup>	LDL cholesterol <sup>181</sup>	rs11206510	Labos, <sup>112</sup> Tragante, <sup>231</sup> Krarup, <sup>104</sup> Mega, <sup>139</sup> Hughes, <sup>83</sup> Bjornsson, <sup>19</sup> Tikkanen, <sup>227</sup> Ripatti, <sup>180</sup> Bolton <sup>21</sup>	T/C
<b>2p11</b>	VAMP5-VAMP8-GGCX	2013	Deloukas <sup>40</sup>	Intracellular vesicle traffic <sup>137</sup>	rs1561198	Krarup, <sup>104</sup> Bjornsson <sup>19</sup>	A/G
<b>2p21</b>	ABCG5-ABCG8	2011	IBC 50K CAD Consortium <sup>87</sup>	LDL cholesterol <sup>181</sup>	rs6544713	Krarup, <sup>104</sup> Bjornsson <sup>19</sup>	T/C
					rs4299376		
<b>2p24</b>	APOB	2013	Deloukas <sup>40</sup>	LDL cholesterol <sup>181</sup>	rs515135	Krarup, <sup>104</sup> Bjornsson <sup>19</sup>	G/A

	Genes involved	Year	Original reference(s)	Function and possible relevance to CAD	Lead SNP and proxy SNPs	Included in risk scores	Minor/major allele
<b>2p24</b>	TTC32-WDR35*	2012	Lu <sup>123</sup>		rs2123536		A/G
<b>2q33</b>	WDR12	2009	Kathiresan <sup>96</sup>	Component of nuclear protein complex <sup>137</sup>	rs6725887	Labos, <sup>112</sup> Tragante, <sup>231</sup> Krarup, <sup>104</sup> Mega, <sup>139</sup> Hughes, <sup>83</sup> Bjornsson, <sup>19</sup> Tikkanen, <sup>227</sup> Ripatti, <sup>180</sup> Bolton <sup>21</sup>	C/T
<b>2q36</b>	Pseudogene ENSG000000197218	2007	Samani <sup>190</sup>		rs2943634		A/C
<b>2p24</b>	AK097927	2013	Deloukas <sup>40</sup>		rs16986953		A/G
<b>2q22</b>	ZEB2-ACO74093.1	2013	Deloukas <sup>40</sup>	ZEB2 transcriptional repressor <sup>137</sup>	rs2252641	Krarup, <sup>104</sup> Bjornsson <sup>19</sup>	G/A
<b>3q22</b>	MRAS	2009	Erdmann <sup>50</sup>	Cell growth and differentiation <sup>137</sup>	rs9818870	Labos, <sup>112</sup> Krarup, <sup>104</sup> Mega, <sup>139</sup> Hughes, <sup>83</sup> Bjornsson, <sup>19</sup> Ripatti <sup>180</sup>	T/C
					rs2306374	Tragante, <sup>231</sup> Tikkanen, <sup>227</sup> Bolton <sup>21</sup>	C/T
<b>3p24</b>	PLCL2**	2014	Hirokawa <sup>74</sup>	Inflammation	rs4618210		G/A
<b>4q31</b>	GUCY1A3	2012	Deloukas, <sup>40</sup> Lu <sup>123</sup>	Hypertension <sup>181</sup>	rs7692387	Krarup, <sup>104</sup> Bjornsson <sup>19</sup>	G/A
					rs1842896		G/T
					rs273909		G/A
<b>4q31</b>	EDNRA	2013	Deloukas <sup>40</sup>	Vasoconstriction <sup>137</sup>	rs1878406	Krarup, <sup>104</sup> Bjornsson <sup>19</sup>	T/C
<b>4q12</b>	REST-NOA1	2015	Nikpay <sup>159</sup>	SMCs in a quiescent state <sup>137</sup>	rs17087335		T/G
<b>5p15</b>	IRX1	2011	Aoki <sup>9</sup>		rs11748327	Tragante <sup>231</sup>	C/T
<b>5q31</b>	IL5	2011	IBC 50K CAD Consortium <sup>87</sup>		rs2706399		G/T

	Genes involved	Year	Original reference(s)	Function and possible relevance to CAD	Lead SNP and proxy SNPs	Included in risk scores	Minor/ major allele
<b>5q31</b>	SLC22A4-SLC22A5	2013	Deloukas <sup>40</sup>	Organic cation transporter <sup>137</sup>	rs273909	Krarp, <sup>104</sup> Bjornsson <sup>19</sup>	C/T
<b>6p21</b>	KCNK5	2013	Deloukas <sup>40</sup>	Potassium channel protein <sup>137</sup>	rs2048327 rs10947789	Hughes <sup>83</sup> Krarp, <sup>104</sup> Bjornsson <sup>19</sup>	A/G T/C
<b>6p21</b>	ANKS1A	2011	Schunkert <sup>198</sup>	HDL cholesterol <sup>181</sup>	rs17609940	Labos, <sup>112</sup> Tragante, <sup>231</sup> Krarp, <sup>104</sup> Mega, <sup>139</sup> Bjornsson, <sup>19</sup> Tikkanen <sup>227</sup>	G/C
<b>6p21</b>	HCG27-HLA-B-HLA-C	2012	Davies <sup>39</sup>		rs12205331 rs2077750	Krarp <sup>104</sup>	T/C G/A
<b>6p21</b>	C6orf10-BTNL2*	2012	Lu <sup>123</sup>		rs3869109		G/A
<b>6p22</b>	BTN2A1**	2011	Yamada <sup>257</sup>		rs9268402		A/G
<b>6q23</b>	TCF21	2011	Schunkert <sup>198</sup>	Transcriptional regulator <sup>137</sup>	rs6929846 rs12190287	Labos, <sup>112</sup> Tragante, <sup>231</sup> Krarp, <sup>104</sup> Mega, <sup>139</sup> Bjornsson, <sup>19</sup> Bolton <sup>21</sup>	T/C C/G
<b>6p24</b>	PHACTR1	2009	Kathiresan <sup>96</sup>	Protein phosphatase 1 activity regulation <sup>137</sup>	rs1967917 rs12526453	Labos, <sup>112</sup> Tragante, <sup>231</sup> Krarp, <sup>104</sup> Hughes, <sup>83</sup> Bjornsson, <sup>19</sup> Tikkanen <sup>227</sup>	A/G C/G
<b>6p24</b>	ADTRP-C6orf105*	2012	Lu <sup>123</sup>		rs9349379 rs1332844	Mega, <sup>139</sup> Ripatti <sup>180</sup> Bolton <sup>21</sup>	C/T C/T
<b>6q25</b>	MTHFD1L	2007	Samani, <sup>190</sup> WTCCC <sup>243</sup>		rs6903956	Tragante <sup>231</sup>	A/G
<b>6q25</b>	SLC22A1-SLC22A3	2009	Tregouet <sup>234</sup>	Lipoprotein(a) <sup>137</sup>	rs6922269 rs2048327	Hughes, <sup>83</sup> Bjornsson <sup>19</sup>	A/G C/T

	Genes involved	Year	Original reference(s)	Function and possible relevance to CAD	Lead SNP and proxy SNPs	Included in risk scores	Minor/major allele
<b>6q25</b>	LPA	2009	Tregouet <sup>234</sup>	LDL cholesterol <sup>181</sup>	rs3798220	Labos, <sup>112</sup> Tragante, <sup>231</sup> Krarup, <sup>104</sup> Mega, <sup>139</sup> Bjornsson, <sup>19</sup> Tikkanen, <sup>227</sup> Ripatti, <sup>180</sup> Bolton <sup>21</sup>	C/T
					rs10455872	Mega, <sup>139</sup> Bjornsson <sup>19</sup>	G/A
					rs3127599	Hughes <sup>83</sup>	A/G
					rs7767084	Hughes <sup>83</sup>	C/T
					rs10755578	Hughes <sup>83</sup>	G/C
<b>6q26</b>	PLG	2013	Deloukas <sup>40</sup>	Fibrinolysis <sup>137</sup>	rs4252120	Krarup, <sup>104</sup> Bjornsson <sup>19</sup>	T/C
<b>7p21</b>	HDAC9	2013	Deloukas <sup>40</sup>	Represses beige adipogenesis <sup>157</sup>	rs2023938	Krarup, <sup>104</sup> Bjornsson <sup>19</sup>	G/A
<b>7q22</b>	7q22-BCAP29	2011	C4D Genetics Consortium <sup>34</sup>		rs10953541	Labos, <sup>112</sup> Tragante, <sup>231</sup> Bjornsson, <sup>19</sup> Tikkanen <sup>227</sup>	C/T
<b>7q32</b>	ZC3HC1	2011	Schunkert <sup>198</sup>	Encodes regulator of cell proliferation <sup>137</sup>	rs12539895	Krarup <sup>104</sup>	A/G
<b>7q36</b>	NOS3	2015	Nikpay <sup>159</sup>	Production of nitric oxide, hypertension <sup>137</sup>	rs11556924	Labos, <sup>112</sup> Tragante, <sup>231</sup> Krarup, <sup>104</sup> Mega, <sup>139</sup> Bjornsson, <sup>19</sup> Tikkanen, <sup>227</sup> Bolton <sup>21</sup>	C/T
<b>8p22</b>	LPL	2013	Deloukas <sup>40</sup>	Lipolysis of lipoproteins <sup>137</sup>	rs3918226		T/C
					rs264	Krarup, <sup>104</sup> Bjornsson <sup>19</sup>	G/A
<b>8q24</b>	TRIB1	2011	IBC 50K CAD Consortium <sup>87</sup>	Triglycerides <sup>181</sup>	rs2954029	Krarup, <sup>104</sup> Bjornsson <sup>19</sup>	A/T
					rs17321515		G/A
					rs10808546		A/G

	Genes involved	Year	Original reference(s)	Function and possible relevance to CAD	Lead SNP and proxy SNPs	Included in risk scores	Minor/ major allele
<b>9p21</b>	CDKN2A- CDKN2B	2007	Helgadottir, <sup>71</sup> McPherson <sup>136</sup>	Vessel wall, cell proliferation and inflammation <sup>92,137</sup>	rs4977574	Labos, <sup>112</sup> Tragante, <sup>231</sup> Mega, <sup>139</sup> Tikkanen, <sup>227</sup> Ripatti <sup>180</sup>	G/A
					rs10757274		G/A
					rs2383206		G/A
					rs3217992		T/C
					rs1333049	Krurup, <sup>104</sup> Bjornsson, <sup>19</sup> Bolton, <sup>21</sup> Hughes <sup>83</sup>	C/G
<b>9q34</b>	ABO	2011	Reilly <sup>175</sup>	Thrombogenesis, myocardial infarction <sup>181</sup>	rs10757278		G/A
					rs579459	Labos, <sup>112</sup> Tragante, <sup>231</sup> Krurup, <sup>104</sup> Bjornsson, <sup>19</sup> Tikkanen, <sup>227</sup> Bolton <sup>21</sup> Mega <sup>139</sup>	C/T
<b>10p11</b>	KIAA1462	2011	C4D Genetics Consortium <sup>34</sup>	Component of endothelial cell-cell junctions <sup>137</sup>	rs9411489		T/C
					rs651007		T/C
<b>10q11</b>	CXCL12- HNRNPA3P1	2007	Samani <sup>190</sup>	Inflammation, neutrophil migration <sup>137</sup>	rs2505083	Labos, <sup>112</sup> Krurup, <sup>104</sup> Bjornsson, <sup>19</sup> Bolton <sup>21</sup>	C/T
					rs3739998	Tragante <sup>231</sup>	C/G
					rs1746048	Labos, <sup>112</sup> Tragante, <sup>231</sup> Mega, <sup>139</sup> Tikkanen, <sup>227</sup> Ripatti, <sup>180</sup> Bolton <sup>21</sup>	C/T
					rs915083		A/C
					rs2047009	Bjornsson <sup>19</sup>	C/T
					rs501120	Krurup, <sup>104</sup> Bjornsson, <sup>19</sup> Hughes <sup>83</sup>	A/G

	Genes involved	Year	Original reference(s)	Function and possible relevance to CAD	Lead SNP and proxy SNPs	Included in risk scores	Minor/major allele
<b>10q23</b>	LIPA	2011	C4D Genetics Consortium, <sup>34</sup> IBC 50K CAD Consortium <sup>87</sup>	Intracellular hydrolysis of cholesterol esters <sup>137</sup>	rs1412444	Labos, <sup>112</sup> Tragante, <sup>231</sup> Krarup, <sup>104</sup> Bjornsson, <sup>19</sup> Tikkanen <sup>227</sup>	T/C
<b>10q24</b>	CYP17A1 - CNNM2-NT5C2	2011	Schunkert <sup>198</sup>	Steroidogenic pathway <sup>137</sup>	rs12413409	Labos, <sup>112</sup> Tragante, <sup>231</sup> Krarup, <sup>104</sup> Mega, <sup>139</sup> Bjornsson, <sup>19</sup> Tikkanen, <sup>227</sup> Ripatti, <sup>180</sup> Bolton <sup>21</sup>	G/A
<b>11q22</b>	PDGFD	2011	C4D Genetics Consortium <sup>34</sup>	Proliferation of SMCs <sup>137</sup>	rs12411886 rs974819	Labos, <sup>112</sup> Tragante, <sup>231</sup> Krarup, <sup>104</sup> Bjornsson, <sup>19</sup> Bolton <sup>21</sup>	A/C A/G
<b>11q23</b>	APOA5 - APOC3 - ZNF259	2011	Schunkert <sup>198</sup>	Triglycerides metabolism <sup>137,181</sup>	rs964184	Labos, <sup>112</sup> Tragante, <sup>231</sup> Mega, <sup>139</sup> Bjornsson, <sup>19</sup> Tikkanen <sup>227</sup>	G/A
<b>11p15</b>	SWAP70	2015	Nikpay <sup>159</sup>	Leukocyte and SMC migration and adhesion <sup>137</sup>	rs9326246 rs10840293	Krarup <sup>104</sup>	G/C A/G
<b>12q24</b>	SH2B3	2009	Gudbjartsson <sup>66</sup>	Negative regulator of cytokine signaling, <sup>137</sup> hypertension <sup>181</sup>	rs3184504	Labos, <sup>112</sup> Tragante, <sup>231</sup> Krarup, <sup>104</sup> Mega, <sup>139</sup> Bjornsson, <sup>19</sup> Tikkanen, <sup>227</sup> Ripatti, <sup>180</sup> Bolton, <sup>21</sup> Hughes <sup>83</sup>	T/C
<b>12q24</b>	HNF1A	2009	Erdmann <sup>50</sup>		rs653178 rs2259816 rs1169313	Labos, <sup>112</sup> Mega, <sup>139</sup> Tikkanen, <sup>227</sup> Ripatti <sup>180</sup>	G/T G/T C/T
<b>12q21</b>	ATP2B1*	2012	Lu <sup>123</sup>	Intracellular calcium homeostasis <sup>137</sup>	rs7136259		T/C



	Genes involved	Year	Original reference(s)	Function and possible relevance to CAD	Lead SNP and proxy SNPs	Included in risk scores	Minor/major allele
<b>12q24</b>	KSR2	2015	Nikpay <sup>159</sup>	Obesity <sup>137</sup>	rs11830157		G/T
<b>13q12</b>	FLT1	2013	Deloukas <sup>40</sup>	Angiogenesis <sup>137</sup>	rs9319428	Krarp, <sup>104</sup> Bjornsson <sup>19</sup>	A/G
<b>13q34</b>	COL4A1-A2	2011	Schunkert <sup>198</sup>	Type IV collagen chain of basement membrane <sup>137</sup>	rs4773144	Labos, <sup>112</sup> Tragante, <sup>231</sup> Krarp, <sup>104</sup> Mega, <sup>139</sup> Bjornsson, <sup>19</sup> Tikkanen, <sup>227</sup> Bolton <sup>21</sup>	G/A
					rs9515203	Bjornsson <sup>19</sup>	T/C
<b>14q32</b>	HHIPL1	2011	Schunkert <sup>198</sup>		rs3809346		C/T
					rs2895811	Labos, <sup>112</sup> Tragante, <sup>231</sup> Krarp, <sup>104</sup> Mega, <sup>139</sup> Bjornsson, <sup>19</sup> Tikkanen, <sup>227</sup> Bolton <sup>21</sup>	C/T
<b>15q25</b>	ADAMTS7	2011	C4D Consortium, <sup>34</sup> Reilly, <sup>175</sup> Schunkert <sup>198</sup>	Proliferative response to vascular injury <sup>137</sup>	rs3825807	Labos, <sup>112</sup> Tragante, <sup>231</sup> Mega, <sup>139</sup> Bjornsson, <sup>19</sup> Tikkanen <sup>227</sup>	A/G
					rs7173743	Krarp <sup>104</sup>	T/C
<b>15q26</b>	FURIN-FES	2013	Deloukas <sup>40</sup>	Hypertension <sup>181</sup>	rs4380028	Tikkanen, <sup>227</sup> Bolton <sup>21</sup>	C/T
<b>15q22</b>	SMAD3	2007	Samani <sup>190</sup>	Mediator of transforming growth factor- $\beta$ signaling <sup>137</sup>	rs17514846	Krarp, <sup>104</sup> Bjornsson <sup>19</sup>	A/C
					rs17228212		C/T
<b>15q26</b>	MFGE8-ABHD2	2015	Nikpay <sup>159</sup>	Neovascularization <sup>137</sup>	rs56062135		C/T
					rs8042271		G/A
<b>17p11</b>	RAI1-PEMT-RASD1	2011	Schunkert <sup>198</sup>		rs12936587	Labos, <sup>112</sup> Krarp, <sup>104</sup> Mega, <sup>139</sup> Bjornsson, <sup>19</sup> Tikkanen, <sup>227</sup> Bolton <sup>21</sup>	G/A
<b>17p13</b>	SMG6-SRR	2011	Schunkert <sup>198</sup>		rs216172	Labos, <sup>112</sup> Tragante, <sup>231</sup> Mega, <sup>139</sup> Bjornsson, <sup>19</sup> Tikkanen <sup>227</sup>	C/G
					rs143499	Krarp <sup>104</sup>	C/T

	Genes involved	Year	Original reference(s)	Function and possible relevance to CAD	Lead SNP and proxy SNPs	Included in risk scores	Minor/major allele
17q21	UBE2Z-GIP	2011	Schunkert <sup>198</sup>	Apoptosis <sup>137</sup>	rs46522	Labos, <sup>112</sup> Tragante, <sup>231</sup> Krarup, <sup>104</sup> Mega, <sup>139</sup> Bjornsson <sup>19</sup>	T/C
17q23	BCAS3	2015	Nikpay <sup>159</sup>	Angiogenesis <sup>137</sup>	rs15563	Krarup <sup>104</sup>	C/T
18q21	PMAIP1-MC4R	2015	Nikpay <sup>159</sup>	Leptin signaling, obesity <sup>137</sup>	rs7212798		C/T
19p13	LDLR-SMARCA4	2009	Kathiresan <sup>96</sup>	LDL cholesterol <sup>181</sup>	rs663129		A/G
19q13	APOE-APOC1	2011	IBC 50K CAD Consortium <sup>87</sup>	LDL cholesterol <sup>181</sup>	rs1122608	Labos, <sup>112</sup> Tragante, <sup>231</sup> Krarup, <sup>104</sup> Mega, <sup>139</sup> Bjornsson, <sup>19</sup> Tikkanen, <sup>227</sup> Ripatti, <sup>180</sup> Bolton, <sup>21</sup> Hughes <sup>83</sup>	G/T
19q13	ZNF507-LOC400684	2015	Nikpay <sup>159</sup>		rs2228671		T/C
19p13	DOT1L-AP3D1-SF3A2**	2015	Hirokawa <sup>74</sup>		rs2075650	Krarup, <sup>104</sup> Bjornsson <sup>19</sup>	G/A
21q22	MRPS6-SLC5A3-KCNE2	2009	Kathiresan <sup>96</sup>	Maintains cardiac electric stability <sup>137</sup>	rs445925	Bjornsson <sup>19</sup>	C/T
22q11	POM121L9P-ADORA2A	2015	Nikpay <sup>159</sup>	Infarct-sparing effects <sup>137</sup>	rs12976411		T/A
					rs35792872	Labos, <sup>112</sup> Tragante, <sup>231</sup> Mega, <sup>139</sup> Bjornsson, <sup>19</sup> Tikkanen, <sup>227</sup> Ripatti, <sup>180</sup> Bolton, <sup>21</sup> Hughes <sup>83</sup>	T/C
					rs7278204	Bolton <sup>21</sup>	G/A
					rs9305545		A/G
					rs180803		G/T

\* Association only in Han Chinese. \*\* Association only in Japanese. SNP indicates single nucleotide polymorphism; WTCCC, Wellcome Trust Case Control Consortium; LDL, low-density lipoprotein; SMC, smooth muscle cell. Data collected from the original articles,<sup>9,34,39,40,50,66,71,74,87,96,123,136,159,175,190,198,234,243,257,19,21,83,104,112,139,180,227,231</sup> from Roberts,<sup>181</sup> and McPherson et al.<sup>137</sup>

## **2.7. Differences in pathophysiology and clinical characteristics between STEMI and NSTEMI**

The MI subtypes STEMI and NSTEMI differ from each other in pathophysiology, in treatment (section 2.5.), and in patient characteristics.

In STEMI, the thrombus is mostly occlusive, sustained, and fibrin-rich, whereas in NSTEMI the thrombus is platelet-rich, only partially occlusive, dynamic, or even absent,<sup>172</sup> and detaching microthrombi may embolize downstream.<sup>11</sup> Furthermore, in NSTEMI, length of plaque rupture is smaller,<sup>229</sup> and plaques are more calcified.<sup>78</sup> As can be suspected from the changes in ECG, infarct size in NSTEMI is smaller.<sup>192</sup> Recurrent MIs are more frequently of the same type as was the first MI.<sup>186</sup>

Patients with STEMI often have angiographically milder disease than do patients with NSTEMI, and are typically younger and more likely to be men and smokers.<sup>17,127</sup> Furthermore, among STEMI patients, diabetes, dyslipidemia, and hypertension are less common than among NSTEMI patients.<sup>127</sup>

Although many characteristics differ between STEMI and NSTEMI patients, no difference has emerged in the genetics of these conditions. Many of the known CAD variants have been analyzed with respect to ACS subtype, with no difference appearing in their frequencies between STEMI and NSTEMI patients.<sup>242</sup>

## **2.8. Prediction of CAD, MI and outcome**

### **2.8.1. Traditional risk-prediction tools**

Several tools have been developed to evaluate risk for CVD. Cardiovascular risk assessment tools predict the likelihood of developing any specified cardiovascular condition during a predefined time period. These tools include scores predicting CAD among a healthy population as well as tools designed for predicting recurrence or mortality risk among patients already suffering from ACS (Table 3).

The best-known and most widely used tool to predict cardiovascular events among healthy people is the Framingham risk score.<sup>255</sup> It estimates the 10-year-risk for CAD by calculating risk points for gender, age, total or LDL cholesterol, HDL cholesterol, blood pressure, diabetes, and smoking. The Framingham score is useful in primary prevention and in showing patients the impact of each life-style-related risk factor.

The Global Registry of Acute Coronary Events (GRACE) risk scores are designed to predict risk for in-hospital death after admission with ACS<sup>61</sup> and risk for a recurrent MI or CVD death within six months after an MI.<sup>47,56</sup> The GRACE scores serve in the initial presentation to distinguish patients most likely to benefit from an invasive strategy. These scores include eight to nine variables such as age, heart rate, systolic blood pressure, creatinine level, severity of heart failure (Killip class), possible cardiac arrest at

presentation, ST-segment deviation, and cardiac enzymes. GRACE scores have been validated by many studies and meta-analyses, showing good discriminatory performance across all types of ACS for short- and long-term prognosis.<sup>36</sup> In the patients undergoing PCI for ACS, however, the GRACE six-month post-discharge score was not as reliable as in patients receiving medical therapy only.<sup>2</sup>

The risk score of the Thrombolysis in Myocardial Infarction Study Group (TIMI)<sup>8</sup> evaluates the risk of patients with UAP/NSTEMI to develop severe recurrent ischemia requiring urgent revascularization, to develop another MI, or to die within a fortnight. The seven predictor variables of TIMI score include age  $\geq 65$  years, presence of at least three CAD risk factors, prior coronary artery stenosis of 50% or more, ST-segment deviation at presentation, at least two episodes of angina in the past 24 hours, prior use of aspirin for at least a week, and elevated cardiac biomarkers. Some of these factors are also included in the GRACE scores. In TIMI, the variables were unweighted, and the high-risk patients were excluded in calculating the score, both actions reducing its discriminatory performance.<sup>13</sup> On the other hand, the TIMI score is easy to use.

The Platelet Glycoprotein IIb/IIIa in Unstable Angina: Receptor Suppression Using Integrilin Therapy (PURSUIT)<sup>20</sup> risk score assesses mortality and a composite event of MI or death within one month. It includes age, sex, high Canadian Cardiovascular Society Angina class in the previous six weeks, admission heart rate, ST-segment depression, and signs of heart failure. The discriminatory performance of the PURSUIT score is evaluated as achieving the same level as the GRACE scores.<sup>258</sup>

The Evaluation of the Methods and Management of Acute Coronary Events (EMMACE)<sup>45</sup> risk score includes age, systolic blood pressure, and heart rate and evaluates the risk of dying within 30 days after acute MI. The Simple Risk Index (SRI) includes age, admission systolic blood pressure, and heart rate and predicts 30-day mortality in STEMI patients.<sup>149</sup> The Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries (GUSTO)<sup>30</sup> score evaluates one-year mortality in 30-day survivors of STEMI and includes age, heart rate, left ventricular ejection fraction, previous infarction, and in-hospital heart failure with pulmonary edema.

Although the end-point, time-frame, and the target group vary from score to score, all these risk scores overlap, and the variables included are mostly the same, although they emphasize different points of view. The complexity of the scores and number of variables varies. Despite the fact that all the scores perform quite well, for example the C-statistic of GRACE score being  $\geq 0.80$  in a large validation study,<sup>36</sup> the actual utilization of the scores in everyday practice remains unclear. To evaluate the risk, however, these tools combine only clinical features and risk factors, not genetic information.

**Table 3.** *Comparison of the clinical risk scores.*

Score	Target group	Population of derivation	End-point	N of variables	Variables included in the scores						
					SBP	HR	Age	ST↑ /ST↓	Cardiac enzymes	Signs of heart failure	Other included factors
GRACE <sup>61</sup>	ACS	Multinational registry	In-hospital death	8	x	x	x	x	x	x	Creatinine, cardiac arrest at admission
GRACE <sup>67</sup>	ACS	Multinational registry	Death within 6 months	9	x	x	x	x	x		No PCI during admission, creatinine, history of previous heart failure or MI.
GRACE <sup>56</sup>	ACS	Multinational registry	Death or MI within 6 months	8	x	x	x	x	x	x	Creatinine, cardiac arrest at admission
TIMI <sup>8</sup>	UAP/ NSTEMI	Randomized controlled trial	14-death, MI, or revascularization	7			x	x	x		At least 3 risk factors, prior coronary stenosis, at least 2 anginal episodes in past 24 hours, use of aspirin in past 7 days
PURSUIT <sup>20</sup>	NSTEMI	Randomized controlled trial	30-day death, death or MI	7	x	x	x	x	x		Gender, Canadian angina class
GUSTO <sup>30</sup>	STEMI	Randomized controlled trial	1-year death in 30-day survivors	5		x	x			x	Ejection fraction, previous infarction
EMMACE <sup>45</sup>	MI	Regional registry	30-day death	3	x	x	x				
SRI <sup>49</sup>	STEMI	Randomized controlled trial	30-day death	3	x	x	x				

SBP indicates systolic blood pressure; HR, heart rate; ACS, acute coronary syndrome; UAP unstable angina pectoris; NSTEMI, non-ST-elevation myocardial infarction; STEMI, ST-elevation myocardial infarction; MI, myocardial infarction; PCI, percutaneous coronary intervention. Data collected from the original articles<sup>8,20,30,45,47,56,61,149</sup> and Bavamia et al.<sup>13</sup>

### 2.8.2. Risk prediction using genetic information

Intensive research has recently focused on genetic risk prediction tools for CAD and MI. Genetic risk scores (GRS) are calculated as the sum or the mean of previously suggested risk variants. A GRS can be unweighted, or it can be weighted, for example with published effect estimates. For the GRSs, the SNPs are chosen from among the risk SNPs, which are established by GWAS with large sample sizes for the disease in question. Use of weighted scores instead of unweighted is supported by previous research.<sup>25</sup> Using an unweighted score would assume that each has an effect of the same size, which does not apply in a multifactorial disease.

The capability of GRSs to predict CAD in a healthy population has been promising, and studies on that subject are emerging. The Atherosclerosis Risk in Communities (ARIC) Study presented a risk score of candidate genes back in 2007;<sup>148</sup> it showed an association with incident CAD in a healthy population. Since then, the variants included in most GRSs are selected from among the results from large GWAS, and, as new risk variants are identified, the number of risk variants included in the GRSs is growing. GRSs including SNPs of genome-wide significant association with CAD have improved CAD prediction over traditional risk factors and family history and helped to identify individuals at high risk for the first CAD event in healthy populations in different geographic areas (Table 4).<sup>21,83,104,180,227</sup> Table 2 shows the SNPs included in these risk scores. In a recent study, a genome-scale GRS of over 49 000 SNPs included additionally SNPs of less than genome-wide significant association with CAD.<sup>1</sup> This GRS improved CAD risk prediction at population level independently of clinical risk factors and the study showed that men in the highest quintile of GRS attained 10% cumulative CAD risk 12 to 18 years earlier than men in the lowest quintile of GRS.

An individual with a genetic predisposition for ACS may also be at high risk for recurrent events, since the medical treatment may be ineffective in reducing genetic risk that led to the initial event.<sup>112</sup> Including either a single SNP or a combination of SNPs in a risk prediction model could help in recognizing individuals at high genetic risk and be helpful in secondary prevention. Among the ACS patients, loci at chromosomes 9p21 (rs1333049),<sup>28</sup> 9q34.2 (rs579459),<sup>242</sup> 1p13.3 (rs599839),<sup>49</sup> and 1q41 (rs17465637)<sup>49</sup> have been linked to subsequent cardiovascular outcomes. Of the SNPs, inclusion of locus 9p21<sup>28</sup> and locus 9q34.2<sup>242</sup> in the clinical risk prediction model for recurrent MI or cardiac death after MI improved the classification. The locus at chromosome 9p21.3 was also associated with better five-year survival of high-risk patients with STEMI.<sup>212</sup> Some of the other known CAD risk SNPs have been tested for association for recurrence separately, but with no linkage to survival.<sup>242</sup>

Results regarding GRSs and recurrent ACS are inconsistent. In a study by Tragante and colleagues, a 30-SNP GRS was significantly associated with recurrence risk in a sex- and age- adjusted model.<sup>231</sup> Perhaps due to the limited number of events, this association was not significant after multivariable adjustments, however.

**Table 4.** *Summary of published GRSs with differing numbers of CAD risk SNPs identified in GWAS.*

Risk score	Year	Population	N	N of events	End-point	Median follow-up time (years)	N of SNPs
<b>Predicting CAD in healthy population</b>							
Ripatti <sup>180</sup>	2010	Healthy	30 725	1 264	CAD	10.7	13
Hughes <sup>83</sup>	2012	Healthy men	4 818	1 736	CAD	18	8-15
Tikkanen <sup>227</sup>	2013	Healthy	24 124	1 552	CVD	12	28
Bolton <sup>21</sup>	2013	Healthy	840	180	CAD	15	27
Krärup <sup>104</sup>	2015	Healthy	6 041	374	CAD	11.6 (mean)	45
Mega <sup>139</sup>	2015	Healthy	48 421	3 477	CAD		27
<b>Predicting recurrent ACS/MI</b>							
Tragante <sup>231</sup>	2013	MI	153	72	Recurrent MI	8	30
Labos <sup>112</sup>	2015	ACS	3 503	389	Composite of all-cause mortality, recurrent MI, cardiac re-hospitalization	1	30
Mega <sup>139</sup>	2015	MI	2 878 +1 999	320+2 29	CAD	4.9 + 2	27
<b>Predicting other CAD related end-points</b>							
Björnsson <sup>19</sup>	2015	CAD patients	8 622		CAD extent		50
Hernesniemi <sup>73</sup>	2015	Autopsy	1 035	306	Sudden death due to CAD		153

*SNP indicates single nucleotide polymorphism; CAD, coronary artery disease; CVD, cardiovascular disease; ACS, acute coronary syndrome; MI, myocardial infarction. Data collected from original articles.<sup>19,21,73,83,104,112,139,180,227,231</sup>*

In a study consisting of several different cohorts, Mega and colleagues reported that higher risk quintiles of 27-SNP GRS were associated with raised risk of both incident and recurrent CAD events.<sup>139</sup> In this study, the follow-up time for recurrence differed across cohorts, the mean values ranging from 2.0 to 4.9 years.<sup>139</sup> The population in these cohorts was highly selected because of its randomized controlled trial study design. The GRS was used as risk quintiles, differing from the practice in other studies. The survival analysis was also conducted separately in statin-user and non-user groups.

In a study of three pooled cohorts comprising 3 503 ACS patients, a 30-SNP GRS was not associated with outcome within one year after ACS,<sup>112</sup> nor did the addition of the GRS to the GRACE risk model improve risk prediction. In this study, the GRS served as a continuous variable, and the patient population was more heterogeneous due to the

study design, when compared to the study by Mega and colleagues.<sup>139</sup> Follow-up time was also limited to one year.

Furthermore, GRSs comprising CAD risk variants have also shown an association with extent of atherosclerosis measured in coronary angiography<sup>19</sup> and in sudden death<sup>73</sup> caused by CAD.

## **2.9. Other manifestations of CVD**

### **2.9.1. Peripheral artery disease**

In PAD, atherosclerosis narrows the arteries typically in the lower extremities and weakens the oxygen supply to the peripheral tissues. Patients with PAD suffer from claudication or critical limb ischemia, but PAD can also present with atypical symptoms or be asymptomatic.<sup>35,217</sup> The diagnosis is based on abnormal ankle brachial index (ABI), since blood flow to the periphery weakens. PAD is associated with morbidity comprising impaired functional capacity, frailty, impaired quality of life, and high medical-care costs. Atherosclerotic PAD is highly prevalent worldwide, affecting more than 200 million people.<sup>54</sup> From 2000 to 2010, the number of patients suffering from PAD increased by almost 30% in low- and middle-income countries and by 13.1% in high-income countries.<sup>54</sup> The prevalence of PAD increases with age, and PAD affects a substantial proportion of the elderly.<sup>35</sup>

The clinical risk factors for CAD and atherosclerotic PAD progression are the same: dyslipidemia, hypertension, smoking and diabetes mellitus. In addition, age is an independent predictor for PAD progression as determined by ABI decline.<sup>97,217</sup> No clear association between PAD and gender exists. However, more severe disease manifestations occur in men.<sup>35</sup> In women, PAD appears more often with atypical pain.<sup>133</sup> Smoking seems to be associated with PAD at an even higher relative risk level than for CAD.<sup>53</sup> PAD is associated with increased morbidity and mortality from incident coronary and cerebrovascular disease.<sup>35</sup>

In contrast to CAD, in which thrombosis in unstable plaque leads to ACS, acute events in PAD are uncommon. Instead, in PAD, the symptoms slowly worsen as the arteries progressively narrow. This signals that both environmental and genetic factors contribute to PAD risk in a somewhat different manner than they contribute to CAD and to ACS. Genetic factors contributing to PAD may act independently of clinical risk factors.<sup>107</sup>

When compared to CAD, fewer genetic markers linked to PAD have emerged. This may result from the greater clinical and genetic heterogeneity of PAD. Additionally, the role of environmental factors is proposed to be greater in PAD than in CAD development.<sup>113</sup> Genetic factors are suggested to affect especially early-onset PAD.<sup>107</sup>

A few SNPs have shown an association with PAD in GWAS.<sup>62,70,102,107,108,154,224</sup> The first identified CAD risk locus 9p21 (rs10757278),<sup>70</sup> a nicotine-dependence related SNP



(rs1051730) in chromosome 15,<sup>224</sup> and a variant linked to abdominal aortic aneurysm in chromosome 9 (rs7025486)<sup>62</sup> contribute to PAD. Additionally, rs1902341<sup>102</sup> near the *OSBPL* gene and rs653178<sup>108</sup> near the *SH2B3-ATXN* gene were associated with PAD. In a meta-analysis, a variant near *PAX2* (rs6584389) associated with PAD ( $P < 1 \times 10^{-6}$ ), but the significance did not achieve genome-wide level.<sup>154</sup> Stemming from the greater heterogeneity, GWASs of larger sample sizes are required to aid in the discovery of new PAD risk variants. Additionally, phenotypic stratification of patients with PAD may be useful in detecting new genetic risk variants, as well.<sup>107</sup>

Although the genetic contribution to PAD is suggested differ from that of CAD/MI, some of the identified PAD risk variants associate also with CAD/MI or with other manifestations of CVD, such as abdominal aortic aneurysms.<sup>62</sup> This indicates that common genetic risk factors are present across the subtypes of CVD, i.e. the risk variants are pleiotropic.<sup>107</sup>

## 2.9.2. Stroke

In stroke, blood flow to a certain area of the brain is decreased due to blockade or spontaneous rupture of blood vessels, which causes a loss of functional brain tissue.<sup>250</sup> This results in symptoms such as trouble with speech, visual loss, sudden weakness, dizziness, or headache.<sup>93</sup> The diagnosis of stroke is primarily based on typical symptoms and CT imaging. If the symptoms are transient, i.e. they last less than 24 hours, the condition is referred to as transient ischemic attack (TIA). Depending on the mechanism, a stroke can be either ischemic or hemorrhagic. An ischemic stroke, 87% of cases,<sup>151</sup> is caused by a lack of blood supply to the small arteries supplying localized areas of the brain. This lack can result from a number of processes, including atherosclerotic small vessel disease causing occlusion and ischemia downstream in the brain, large vessel disease causing occlusion or emboli, cardioembolic stroke, or from infrequent mechanisms such as cervical artery dissections.<sup>163</sup> In hemorrhagic stroke, 13% of cases,<sup>151</sup> a vessel supplying the brain ruptures and blood accumulates in either the sub-arachnoid space or intra-cerebral space of the brain.

Annually 15 million people suffer from stroke.<sup>151</sup> In relation to the treatment and prevention of traditional risk factors, the stroke incidence has decreased by 42% in high-income countries during the last four decades. However, in low- and middle-income countries, stroke incidence has increased rapidly.<sup>51</sup> As in the case of CAD and PAD, stroke is also associated with functional impairment, impaired quality of life, and high health-care costs. As many as one-fourth of stroke patients become dependent on receiving help to function in their everyday lives, and almost half suffer from cognitive deficits.<sup>151</sup>

As in CAD and PAD, the traditional risk factors, such as sex, smoking, hypertension, diabetes, and dyslipidemia greatly affect the risk for stroke. Two-thirds of strokes are first events, thus the prevention and treatment of risk factors is crucial.<sup>141</sup> Stroke is noticeably more frequent among women than among men. Hypertension affects stroke risk more than CAD or PAD risk.<sup>151</sup>

Not all strokes, however, can be explained by risk factors alone, but, as in the other manifestations of atherosclerosis, the genetics also matter.<sup>170</sup> Up to 50% of an individual's stroke risk is evaluated to depend on genetics, but the heritability estimates depend on age, sex, and stroke subtype.<sup>230</sup> In addition to genetic variants linked to certain risk factors, many other genetic variants affect the risk for stroke.<sup>64,65,67,77,88,99,171,232,233,254</sup> For example, common variants previously linked to atrial fibrillation, 4q25 near *PITX2* and 16q22 near *ZFHX3*, have been associated with ischemic stroke.<sup>65,67</sup> Some of the risk variants for ischemic stroke are pleiotropic and additionally confer the risk for CAD. Among these are the first discovered CAD risk variant on chromosome 9p21<sup>64</sup> and the MI-linked variant at the *ABO* locus.<sup>254</sup> Moreover, a large meta-analysis analyzed the known CAD risk variants for association with ischemic stroke, particularly large artery stroke, and vice versa, and reported several additional shared genetic risk variants.<sup>44</sup>

### **3. Aims of the study**

The main aims of this study were to evaluate the importance of clinical risk factors and genetics in predicting the recurrence and prognosis of ACS and its subtypes. More precisely, the objectives were as follows.

- 1) To collect a cohort of consecutive patients undergoing coronary angiography in Helsinki University Central Hospital, to describe their demographics, angiographic findings, and the stability of their CAD and to analyze their risk factors and the frequency of revascularization (I)
- 2) To test the ability of a GRS in predicting recurrent ACS and CAD mortality (II)
- 3) To determine the differences between NSTEMI and STEMI patients in clinical factors and genetics (III)
- 4) To assess whether the genetic variant discovered in Study III is associated with adverse outcome, cardiovascular risk factors, or other manifestations of atherosclerosis, such as PAD (IV)

## 4. Methods

### 4.1. Design of the substudies

#### *Study I*

An observational cohort study of consecutive coronary angiography patients purposed to evaluate their risk-factor profiles, disease stability, and severity, as well as the revascularization strategy.

#### *Study II*

A prospective, observational cohort study to analyze the capability of GRSs to predict recurrent ACS and CAD mortality.

#### *Study III*

A GWAS to search for risk variants exclusively for STEMI/NSTEMI in a case-control setting. The finding was replicated in another case-control cohort and in a prospective cohort.

#### *Study IV*

A prospective, observational cohort study to evaluate outcome, cardiovascular risk factors, and other manifestations of atherosclerosis in patients with or without risk alleles on chromosome 1p13.3 (rs656843).

## 4.2. Study cohorts

The basis of Studies I to IV is the Corogene cohort. All methods and results are from the Corogene study, unless otherwise indicated. Patients from the Tampere Acute Coronary Syndrome Study (TACOS) and FINRISK cohorts are included in Studies III to IV to further test the discoveries of the Corogene Study (Table 5). Selected individuals from FINRISK cohorts served also as controls. Participants in all of these cohorts gave their written informed consent. The Ethics Committee of Helsinki University Hospital, Internal Medicine approved the research protocol for the Corogene study. The Ethics Committee of the National Institute of Welfare and the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District approved the protocols of the FINRISK studies. The Ethics Committee at Tampere University Hospital approved the TACOS protocol.

**Table 5.** *Individuals included in Studies I-IV.*

	<b>Corogene</b>	<b>FINRISK</b>	<b>TACOS</b>
<b>N</b>	5 294	23 036	998
<b>Study I</b>	5 294		
<b>Study II</b>	2 090 with ACS		
<b>Study III</b>			
Discovery sample: Corogene	1 863 with MI of which 1579 genotyped	1 576 matched controls	
Replication I: TACOS		566 matched controls	564 genotyped MI patients
Replication II: FINRISK		16 627 of which 848 with MI	
<b>Study IV</b>			
Discovery sample: Corogene	1 768 genotyped ACS patients		
Replication: FINRISK		21 161 of which 439 with PAD	

*TACOS indicates the Tampere Acute Coronary Syndrome Study; ACS, acute coronary syndrome; MI, myocardial infarction; PAD, peripheral artery disease.*

#### **4.2.1. Corogene (I-IV)**

The basis of this study was the Corogene cohort, which comprised 5 294 consecutive angiography patients in Helsinki University Central Hospital between June 2006 and March 2008. The Hospital District of Helsinki and Uusimaa serves more than 1.5 million inhabitants. In the hospitals of this area, a total of over 4800 coronary angiograms are performed annually, and Helsinki University Central Hospital performs 75%.

After excluding patients with other than Finnish citizenship, repeated angiograms for patients already included in the study, and patients with low hemoglobin, recent blood transfusion, or previous heart transplantation (511, 8.8%), the cohort comprised 5 294 patients (91.2% of all angiography patients). The patients were treated with standard drug regimens and procedures.

##### ***Patient classification***

According to the angiography findings and the symptoms, these patients were classified into four groups:

- Group 1. No CAD in angiographic evaluation. The angiogram in these patients was required to reveal either normal or mildly (up to 50%) obstructed coronary arteries. (I)
- Group 2. Stable CAD. The requirement was significant stenosis (more than 50%) in at least one coronary artery and stable symptoms. (I)
- Group 3. ACS. Criteria for inclusion were chest pain typical for ischemia combined with typical ECG changes, biomarker measurements (TnT, CKMBm), and at least one significantly (more than 50%) obstructed coronary artery. (I to IV)
- Group 4. Other ischemic events. Patients had elevated biomarkers, symptoms typical for ACS, and possible ECG changes, but no significant coronary artery stenosis. (I)

##### ***Data collection***

The patient questionnaire provided information on height, symptoms of CAD, other CVD manifestations, and other diseases. Additionally, collected information covered family history for CAD, medications, smoking, alcohol intake, drug abuse, allergies, special diets, social situation, and home nursing. Current smoking was defined as smoking or having quit smoking within six months before study inclusion. Ex-smoking was considered as having quit smoking more than six months before the inclusion.

Patient records, a cardiological data log, and the cardiac-surgery database provided information on height, risk factors, prior cardiac disease and operations, comorbidities, medication, chest x-ray, ECG, echocardiography, angiography, PCI, and CABG. Hypertension, dyslipidemia, and diabetes (types 1 and 2) were defined as fulfilling the criteria for the medication for these disorders.

Blood samples for serum and plasma analyses were drawn from the arterial line during the angiography. Laboratory values collected included complete blood count, CRP, blood lipids, glucose, HbA1c, liver enzymes, bilirubin, activated partial thromboplastin time, renal values (urea, creatinine), electrolytes, CK, cardiac markers

such as b-type n-terminal natriuretic propeptide (proBNP), TnT, CKMBm, and D-dimer. Blood samples additionally served for extraction of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Glomerular filtration rate (GFR) for each patient was estimated from serum creatinine by the Modification of Diet in Renal Disease Study (MDRD) equation.<sup>114</sup>

Patients of the Corogene Study were followed up until death or by the end of the year 2012. Statistics Finland (Tilastokeskus) provided the mortality data. The National Hospital Discharge Registry provided the information on ACS recurrence rate, and incidence of PAD, cerebral infarction, and atherosclerotic aortic disease.

#### **4.2.2. TACOS (III)**

The TACOS Study comprises ACS patients admitted to Tampere University Hospital between January 2002 and March 2003.<sup>160</sup> The diagnostic criteria for ACS in the TACOS and Corogene studies were analogous, except for the use of TnI (TnI>0.2 mg/L) in TACOS whereas Corogene used TnT and CKMBm. Patients transferred from another department within the hospital or initially treated for ACS in other hospitals were excluded. The study sample comprised 998 MI patients, of whom 343 had STEMI, and 655 had NSTEMI. Of these, 564 MI patients had adequate clinical and adequate genotypic information for analysis in Study III.

#### **4.2.3 FINRISK (III-IV)**

FINRISK is a repetitive cohort study for which a new cohort is collected once every five years. The FINRISK cohorts reveal trends in cardiovascular risk factors in Finland.<sup>237,238</sup> A random sample of 25- to 74-year-old inhabitants, representing different regions, genders, and age-groups, participated in each cohort by invitation. Altogether 23 036 individuals took part in the FINRISK 1992, 1997, 2002, and 2007 cohorts.

FINRISK cohorts provided both cases and controls for Studies III and IV. Additionally, the gene expression levels were tested from 513 individuals recruited during 2007 for the Dietary, Lifestyle, and Genetic determinants of Obesity and Metabolic syndrome (DILGOM) study, an extension of the FINRISK 2007 study. Of these samples, 372 served also as controls for the Corogene MI cases in the GWAS in Study III.

In Study III, controls for the Corogene MI patients (discovery sample) were selected from among participants living in the Helsinki-Vantaa region from the FINRISK 1997, 2002, and 2007 studies by use of risk set sampling. Independently for each Corogene MI case, two sex- and birth cohort (+/- 5 years) -matched controls were chosen from all FINRISK participants. Individuals were suitable as controls if they were free of CVD at the time of ACS diagnosis of the chosen matched case. One FINRISK participant could serve as a control several times. For TACOS MI patients (replication sample I), controls came from the FINRISK 1992, 1997, 2002, and 2007 cohorts. The controls were required to be born in Pirkanmaa to match the geographical region of the cases in TACOS.

FINRISK participants with an MI diagnosis in The National Hospital Discharge Registry and with their genotype data available were the selected cases for replication sample II. All the other genotyped FINRISK patients were included in the analysis of prospective setting, with the exception of participants with prevalent MI, insufficient data on phenotype, or who were among the individuals serving as controls in the discovery or in replication sample I. Replication sample II eventually comprised 16 627 participants, with 163 cases of incident NSTEMI, 99 cases of incident STEMI and 222 cases of incident MI with unspecified ST-segment status.

Study IV included FINRISK participants with both genotype and PAD phenotype information available, the individuals with incident PAD serving as cases and other subjects as controls, with the exception of prevalent PAD cases. The National Hospital Discharge Registry provided the information on prevalence and incidence of PAD.

### **4.3 Definitions of study end-points**

#### ***Total mortality, CVD mortality, and CAD mortality (II, IV)***

CVD mortality was considered as death from either CAD, cerebral infarction, PAD, or aortic aneurysm or dissection. CAD-related death was defined according to the International Classification of Diseases (ICD-10) codes I20-I25, I46, R96, or R98 as the direct or as the underlying cause of death, or I21-I22 as the contributing cause of death. Death from cerebral infarction was considered as ICD-10 codes I63-I64 and death from aortic aneurysm as I71. PAD-related death was defined as I70.2, I73.9, and vascular complications of diabetes E10.5, E11.5, E12.5, E13.5, or E14.5. The definition for CAD-related death and use of the Causes of Death Registry from Statistics Finland have been validated.<sup>162</sup>

#### ***Recurrent ACS (II, IV)***

Recurrent ACS was defined as I20.0, I21, or I22 as the main or as the additional diagnosis. Events occurring 28 days after initial hospitalization for ACS were considered in the Corogene cohort as recurrent events. In Study II, a composite event of recurrent ACS or CAD death was formed. In Study IV, CAD-related cause of death was considered as recurrent ACS.

#### ***ACS subtype (III)***

The ACS patients were classified into groups of UAP, NSTEMI, and STEMI according to changes in ECG and release of cardiac biomarkers. The criteria for classification were uniform with the international ACS definitions (section 2.2) supplemented with the criterion of >50% obstruction of at least one coronary artery. In patients with typical STEMI symptoms, ECG, and biomarker release (CKMBm more than 50 mg/l), but with no coronary obstruction, the criterion of significant stenosis observed in the angiography was discarded; these patients were still classified as having STEMI. Study III included only patients with either STEMI or NSTEMI.



#### ***Other manifestations of CVD (IV)***

PAD was defined as I70.2, I73.9, or vascular complications of diabetes E10.5, E11.5, E12.5, E13.5, or E14.5. In the Corogene cohort, PAD was defined as incident, if no claudication symptoms were reported at study inclusion. Cerebral infarction was classified as ICD-10 codes I63-164, and atherosclerotic aortic disease, including aneurysms and dissections, as code I71.

## **4.4 Genotyping**

#### ***Corogene (I-IV)***

The Corogene study participants were genotyped at the Wellcome Trust Sanger Institute, Cambridge, United Kingdom, with the Illumina HumanHap 610-Quad SNP Array. Exclusion criteria for individuals were as follows: a low genotyping success rate < 95%, differing reported and genotype-determined gender, and excess relatedness. Additional exclusion criteria for SNPs were a genotyping success rate < 95%, low MAF < 1%, or  $P < 10^{-6}$  for an exact test of Hardy-Weinberg equilibrium. Only autosomal SNPs were included.

The dataset was completed by genotype imputation using IMPUTE v2.2.2<sup>82</sup> with the 1000 Genomes Project integrated variant set. Uncommon variants (MAF < 5%) and variants with imputation score < 0.6 were excluded. For the GWAS in Study III, any SNPs with  $P < 0.05$  for Pearson's  $X^2$  test for genotype missingness between cases and controls were excluded.

In total, genetic information was available for 1 768 ACS patients, including 1 579 MI cases (962 NSTEMI, 614 STEMI, 3 MI with unspecified ST-segment status).

#### ***FINRISK (III-IV)***

The FINRISK subjects were genotyped with the Affymetrix 6.0 (330), Illumina 610K11 (1 845), HumanCoreExome (19 910), and OmniExpress (2 542) genotyping arrays. Exclusion criteria were the same as in the genotyping of the Corogene cohort. The data were imputed with IMPUTE v2.3.213 with 1000 Genomes Phase 3 haplotypes, which was supplemented with a haplotype set of 2 000 Finnish haplotypes.

#### ***TACOS (III)***

The MI patients of the TACOS cohort were genotyped by standard procedures at the Institute of Molecular Medicine Finland (FIMM), Helsinki, Finland, with the Sequenom MassARRAY iPLEX platform. A total of 74 samples were genotyped twice to ensure the quality of genotyping, and concordance was 100%. Of the TACOS cohort, 390 patients with NSTEMI and 174 patients with STEMI were successfully genotyped.

#### ***Gene expression (III)***

Gene expression was examined from leukocytes of the participants with the Illumina HT-12 expression array.<sup>89</sup> All of the samples were analyzed twice. The correlation between the two measurements was examined by measuring the Pearson's product moment

correlation coefficient (P) and Spearman's rank correlation coefficient ( $\rho$ ) from normalized values. Poor correlation ( $P < 0.94$  or  $\rho < 0.60$ ) between the paired samples led to exclusion.

## 4.5. Statistical methods

### 4.5.1. Statistical tests

Numbers are presented as mean  $\pm$  standard deviation (SD) for normally distributed scale variables and as median [interquartile range (IQR)] for other continuous variables. The number and percentage is shown for each categorical variable. Hazard ratios (HR) and odds ratios (OR) accompanied by 95% confidence intervals (CI) are reported. Chi-square tests determined the statistical difference between groups for categorical variables. Student's t-test for independent samples served to analyze normally distributed scale variables, and the Kruskal-Wallis test to compare other continuous variables. A  $P < 0.05$  denoted the limit for statistical significance.

Cox regression analysis served for survival analysis of longitudinal data in Studies II to IV, and logistic regression analysis for analysis of cross-sectional data, including associations between genetic variants and MI phenotypes. All models were first conducted as age- and sex-adjusted and then, if appropriate, multivariable adjusted. An additive genetic model was assumed for all association tests in Studies II to IV. The Hosmer-Lemeshow goodness-of-fit test determined calibration of the logistic regression models. The proportional hazards assumption was met and was analyzed with use of 'cox.zph' function<sup>60,222,223</sup> of the "survival" package for R v3.2.2 with  $P > 0.05$  for all end-points tested. Linear regression served for studying associations between genetic variants and gene expression levels, with probe intensities set as the dependent variables.

Multivariable adjustment in Study II comprised history of hypertension, dyslipidemia, diabetes, or prior MI, smoking, subtype of ACS, 3-vessel disease, and any congestion observed in chest radiography in addition to age and sex. Interaction terms for interactions between genetic and clinical variables were tested in the multivariable models, but no interactions achieved significance.

In Study III, the Cox proportional hazards analysis was stratified by geographical region, the year and the genotyping batch of the cohort; sex, systolic blood pressure, blood pressure medication, total cholesterol, HDL cholesterol, current smoking, and prevalent diabetes served as covariates. In the case-control setting in Study III, all the MI patients, and STEMI and NSTEMI patients as groups, were contrasted with controls with age, gender, and the 10 first genomic principal components as covariates. Since in replication sample I the controls were matched only for geographical area, not for age or sex, these also could not serve as covariates in the analysis.<sup>164</sup>

Multivariable adjustment in Study IV included age, sex, hypertension, dyslipidemia, diabetes, smoking, 3-vessel disease, and NSTEMI in the Corogene cohort; and age, sex, hypertension, dyslipidemia, diabetes, smoking, and the genotyping cohort and year of the

cohort in the FINRISK study. No interactions appeared in the multivariable models in Study IV. The 10 first genomic principal components were included in the Cox regression analyses, but they showed no significance in the analysis, and thus they were not included in the final multivariable analyses.

The 87 046 directly genotyped SNPs in approximate linkage equilibrium allowed calculation of the genome-wide principal components for the MI patients of the Corogene study with EIGENSTRAT v4.2.<sup>167</sup> The principal components served in Studies III to IV. Inverse-variance weighted fixed- and random-effects meta-analysis models served in combining the results of the logistic regression models with GWAMA v2.1.<sup>124</sup> The Bayesian model comparison analysis method<sup>15</sup> served in comparison of association statistics between MI subtypes.

The statistical analysis tool was IBM SPSS 20.0 for baseline factors in Studies I to IV, for all logistic regression models and Cox regression models including single SNPs in Study II, and for all Cox regression models in Study IV. In Study III, PLINK v1.07<sup>168</sup> was the tool for analyzing directly genotyped variants and SNPTEST v2.4.0<sup>129</sup> for the imputed variants. SNPTEST v2.4.0 additionally allowed investigation of associations between genetic variants and gene expression levels. The survival package<sup>223</sup> for R<sup>173</sup> v2.15.1 (II) and v3.0.2 (III) served as the tool for Cox proportional hazards models with GRSs in Study II and for all Cox proportional hazards models in Study III.

#### **4.5.2. Calculation of GRS**

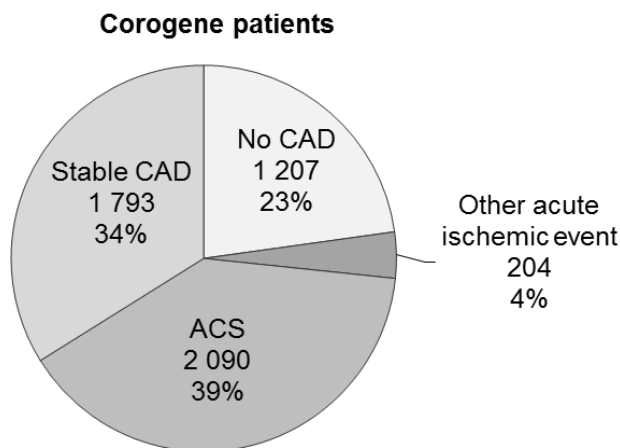
The 153 SNPs reported in the CARDIOGRAMplusC4D Study<sup>40</sup> were chosen to be combined as GRSs in Study II. They were first evaluated separately in age- and sex-adjusted Cox proportional hazards models. End-points considered were recurrent ACS, CAD death, and a composite of recurrent ACS and CAD death. After Bonferroni correction for multiple comparisons, the corrected P-value threshold for statistical significance was  $0.05/153=3.27\text{E-}4$ .

These 153 SNPs were then combined to form three different, partially overlapping GRSs. GRS32 comprised 32 SNPs already discovered even before the CARDIOGRAMplusC4D Study. GRS47 included the SNPs in the GRS32, supplemented by the risk variants that the CARDIOGRAMplusC4D Study uncovered in 2013. GRS153 combined all these 153 SNPs, consisting of both established risk variants and a broader set of independent variants ( $r^2<0.2$ ) strongly associated with CAD at a 5% false discovery rate (FDR) threshold. The GRS values for each individual were calculated as a weighted mean by summing the alleles of each variant, weighting them with the effect sizes reported in the CARDIOGRAMplusC4D Study and dividing by the number of variants included.

Evaluation of clinical value of the GRS involved receiver operating characteristics (ROC) curves and area under the curve (AUC) calculation (survival package of R v2.12.1).

## 5. Results

### 5.1. Overall patient characteristics in the Corogene cohort (I)



**Figure 4** *Patients in the Corogene study. CAD, coronary artery disease; ACS, acute coronary syndrome.*

Figure 4 shows the distribution of the 5 294 patients among the study groups. The most common reason for a coronary angiography during the study period was ACS, and diagnosing stable CAD was the second most common. Of the 2 090 ACS patients, 227 (10.9%) had UAP, 1 134 (54.3%) NSTEMI, and 729 (34.9%) STEMI (II). Almost one-fourth of the patients (1207, 23%) showed no signs of obstructive CAD in the coronary angiography. These patients suffered from stable or atypical chest pain caused by valvular disease, cardiomyopathy, or conditions other than CAD. Coronary angiography was essential to exclude coronary obstruction as a cause for their chest pain symptoms. The patients evaluated as having an acute ischemic event other than ACS suffered from conditions such as type 2 MI, myocarditis, or Takotsubo cardiomyopathy.

The mean age of the cohort was 65.6 years, and 63.8% of the patients were male. These patients with a greater number of diseased coronary arteries were older and more likely to be men (Table 6) (S.V. et al., unpublished results). They were also more likely to suffer from other manifestations of atherosclerosis such as TIA, stroke, or claudication, and to have undergone prior revascularization procedures (PCI or CABG). Furthermore, the prevalence of traditional risk factors rose along with the number of diseased coronaries.

Of the risk factors, diabetes mellitus and smoking were more frequent among male patients. In contrast, female patients were more likely to be hypertensive; dyslipidemia was equally common in both genders. The finding of no obstructive CAD in the coronary

angiography was much more frequent in women than in men. In evaluation by age group, the prevalence of hypertension, dyslipidemia, and diabetes increased with age, but smoking was more common among younger patients undergoing angiography (I, supplementary material).

**Table 6.** *Patient characteristics by disease severity.*

	All patients	CAD severity by coronary angiography				
		No CAD	<50% obstruction	1VD	2VD	3VD
Number of patients	5 294	902	522	1 360	1 037	1 469
Gender (men)	3 379 (63.8)	421 (46.7)	250 (47.9)	897 (66.0)	718 (69.2)	1 091 (74.3)
Age (years)	65.6 ± 11.1	60.2 ± 11.1	66.2 ± 9.8	63.4 ± 11.2	66.9 ± 10.9	69.6 ± 10.0
Body mass index (kg/m <sup>2</sup> )	27.4 ± 4.8	27.1 ± 5.0	27.3 ± 5.4	27.3 ± 4.8	27.5 ± 4.6	27.4 ± 4.5
Hypertension	3 515 (66.4)	493 (54.7)	361 (69.2)	853 (62.7)	732 (70.6)	1 074 (73.1)
Dyslipidemia	3 721 (70.3)	465 (51.7)	362 (69.5)	927 (68.3)	805 (77.8)	1 160 (79.1)
Diabetes	1 054 (19.9)	71 (7.9)	94 (18.0)	209 (15.4)	235 (22.7)	445 (30.3)
Smoking	3 160 (59.7)	431 (47.8)	300 (57.9)	842 (62.1)	668 (65.0)	918 (63.0)
Arrhythmia	1 398 (26.4)	358 (39.7)	184 (35.2)	248 (18.2)	225 (21.7)	381 (25.9)
Prior MI	1 094 (20.7)	37 (4.1)	46 (8.8)	209 (15.4)	267 (25.8)	535 (36.6)
Cardiomyopathy	263 (5.0)	117 (13.1)	42 (8.1)	37 (2.8)	25 (2.5)	41 (2.9)
<b>Other CVD symptoms</b>						
Claudication	537 (10.1)	41 (4.6)	65 (12.5)	74 (5.5)	118 (11.5)	237 (16.2)
Stroke/ TIA	662 (12.5)	86 (9.6)	67 (12.8)	146 (10.8)	127 (12.3)	236 (16.2)
<b>Prior cardiovascular operations</b>						
Prior PCI	671 (12.7)	1 (0.1)	11 (2.1)	153 (11.3)	205 (19.8)	301 (20.5)
Prior CABG	500 (9.4)	0	2 (0.4)	12 (0.9)	68 (6.6)	418 (28.5)
Other	417 (7.9)	65 (7.2)	46 (8.8)	47 (3.5)	81 (7.8)	178 (12.1)
<b>Prior vascular operations</b>						
Lower extremity	184 (3.5)	5 (0.6)	19 (3.7)	15 (1.1)	48 (4.7)	97 (6.6)
Other	106 (2.0)	9 (1.0)	9 (1.7)	14 (1.0)	16 (1.6)	58 (4.0)

*Mean ± standard deviation is shown for continuous variables (age and body mass index) and number (%) for categorical variables (all others). Percentage is calculated of those with information available in each group. CAD indicates coronary artery disease; 1VD, 1-vessel disease; MI, myocardial infarction; CVD, cardiovascular disease; TIA, transient ischemic attack; PCI, percutaneous coronary intervention; CABG, coronary artery by-pass grafting.*

During the study hospitalization or electively after the discharge, 681 (38%) patients with stable CAD underwent PCI and 377 (21%) underwent CABG (Table 7) (S.V. et al., unpublished results). Of the ACS patients, the proportion undergoing PCI was almost double (1484, 71%). CABG was performed for 312 (15%) patients with ACS. No revascularization procedures were performed for patients with other acute ischemic events or without coronary obstructions <50% of lumen diameter. Of all 5 294 patients, 2 854 (54%) were revascularized.

**Table 7.**        *The revascularization of the Corogene patients.*

	<b>Total</b>	<b>All revascularized</b>	<b>PCI</b>	<b>CABG</b>
All patients	5 294	2 854 (53.9)	2 165 (40.9)	689 (13.0)
All patients with CAD/ACS	3 883	2 854 (73.5)	2 165 (55.8)	689 (17.7)
ACS	2 090	1 796 (85.9)	1 484 (71.0)	312 (14.9)
Stable CAD	1 793	1 058 (59.0)	681 (38.0)	377 (21.0)
No CAD/ Other ischemic event	1 411	0	0	0

*Number (% of the total in each row) is shown for all. ACS indicates acute coronary syndrome; CAD, coronary artery disease; PCI, percutaneous coronary intervention; CABG, coronary artery by-pass grafting.*

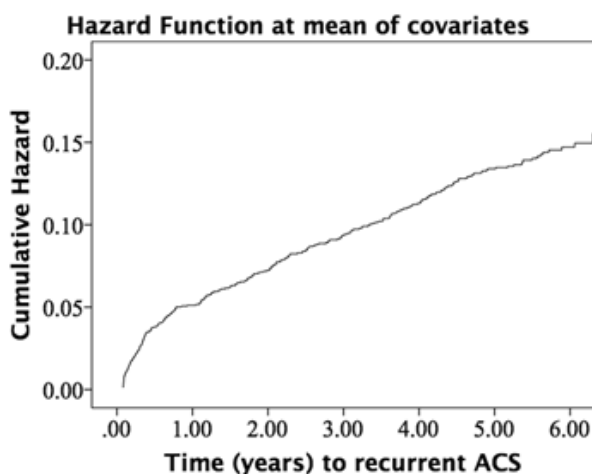
## 5.2. ACS patients (II)

### 5.2.1. Recurrent ACS during follow-up

Of all 2 090 ACS patients, 263 (12.6%) had recurrent ACS, and 473 (22.6%) experienced a composite event of recurrent ACS or CAD death. The recurrent ACS presented within a median 595 days (IQR 130-1 284) and the composite event of recurrent ACS or CAD death within a median 428 days (IQR 63- 1 134) (Figure 5). In most cases, the type of recurrent ACS was NSTEMACS (89 NSTEMI, 95 UAP, 58 STEMI). In 21 cases, information on the recurrent ACS type was unavailable.

Of the clinical risk factors, older age, female gender, history of hypertension, and diabetes mellitus were associated with recurrent ACS (II). Also greater atherosclerotic burden, indicated by 3-vessel disease in coronary angiography, prior MI, previous procedures of PCI or CABG, were linked to higher risk of ACS recurrence. They also more often used medication linked to difficult symptoms, such as long-acting nitrates and diuretics. Patients with recurrent ACS were slightly less frequently on statin therapy than were those without recurrent events. Those with recurrent ACS were more likely to have either NSTEMI or UAP as the index ACS.

The same features that were associated with recurrent ACS were also associated with the composite event of recurrent ACS and CAD death, in addition to higher heart rate, higher CRP, pulmonary congestion, and PCI during initial hospitalization (II, supplementary material). These patients also less often received low-molecular-weight heparin during their initial hospitalization, and less often used clopidogrel, but more often warfarin.



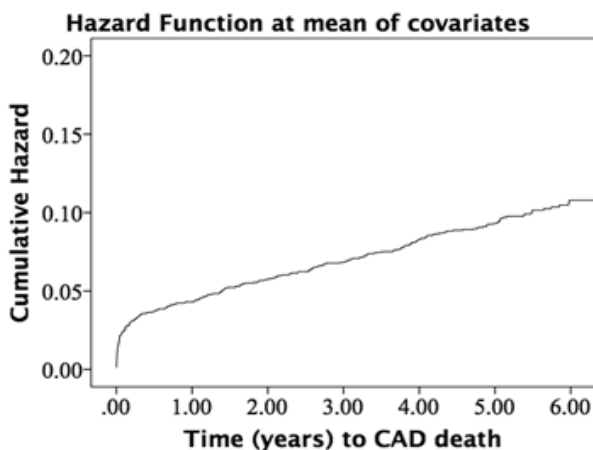
**Figure 5** Cumulative hazard curve for recurrent ACS, age- and sex-adjusted. ACS indicates acute coronary syndrome.

### 5.2.2. Mortality of the ACS patients

In all 2 090 ACS patients, median follow-up time was 1 964 days (IQR 1 542- 2 169), and of these patients, the 1 635 survivors had a median follow-up time of 2 076 days (IQR 1 929-2 231). A total of 455 (21.8%) died within a median 406 (IQR 44-874) days after study inclusion; the figure for 30-day mortality was 71 (3.4%) and 1-year mortality was 156 (7.5%).

Of the non-survivors, 357 (17.1%) had a CVD-related cause of death, and 259 (12.4 %) had a CAD-related cause of death. Median time to CVD death was 331 days (IQR 14-829) and to CAD death 419 (IQR 23- 1 200). Figure 6 shows the hazard curve for CAD mortality, adjusted for age and sex.

Clinical factors associated with CAD death were similar to those associated with recurrent ACS: older age, female gender, history of hypertension or diabetes mellitus, indicators of greater atherosclerotic burden, arrhythmias, and pulmonary congestion. Of the medications, warfarin, diuretics, long-acting nitrates, AT-receptor blockers, and calcium antagonists were associated with CAD mortality. Conversely, PCI or CABG during study hospitalization, and medical treatment with statins, clopidogrel, aspirin, ACE-inhibitors, and low molecular-weight heparin were associated with longer survival (S.V. et al., unpublished results).



**Figure 6** Cumulative hazard curve for CAD-related death, age- and sex-adjusted. CAD indicates coronary artery disease.



### 5.2.3. Single SNP associations with outcome and with risk factors in ACS patients

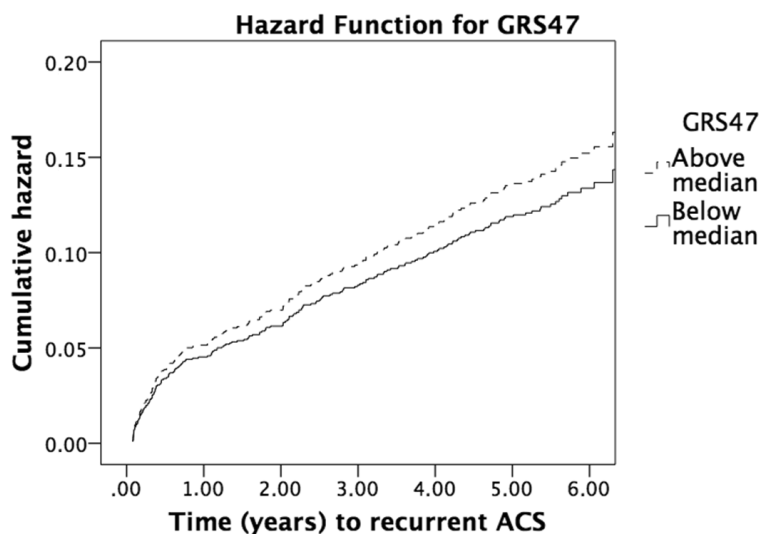
All the 153 SNPs with either genome-wide significant or suggestive association with CAD were tested for associations for recurrent ACS, CAD death, and the composite event of recurrent ACS and CAD death in age- and sex-adjusted Cox models (II, supplementary material). Of all these SNPs, 10 associated nominally with recurrent ACS, eight with CAD death during the follow-up period, and seven with the composite event. After Bonferroni correction, only locus rs2832227 in chromosome 21 near the *TAKL1* gene was significant (HR 1.509, 95% CI 1.160-1.964,  $P=0.002$  for recurrent ACS; HR 1.468, 95% CI 1.202-1.792,  $P<0.001$  for the composite event). After multivariable adjustment for age, sex, history of hypertension, dyslipidemia, diabetes, prior MI, smoking, subtype of ACS, 3-vessel disease, and pulmonary congestion, this locus remained significant (HR 1.591, 95 % CI 1.204-2.101,  $P=0.001$  for recurrent ACS; HR 1.459, 95 % CI 1.176-1.810,  $P=0.001$  for the composite event).

The loci for 9p21 (rs1333049), 1q41 (rs17465637), and *ABO* (rs495828) were associated with none of the end-points.

The 47 SNPs with genome-wide significant associations with CAD were tested for associations with hypertension, dyslipidemia, diabetes mellitus, and smoking. Before Bonferroni correction, four SNPs associated with hypertension (rs12205331, rs11191447, rs9326246, rs9982601), five with dyslipidemia (rs11206510, rs602633, rs12205331, rs1122608, rs1561198), two with diabetes mellitus (rs974819, rs9982601) and six with smoking (rs2048327, rs4773144, rs9515203, rs2895811, rs2954029, rs9319428). Of these SNPs, only rs2895811, near the *HHIPL1* gene, was significant after Bonferroni correction (OR 0.778, 95% CI 0.671-0.902,  $P=0.001$ ) (II, supplementary material).

### 5.2.4. GRS associations with outcome in ACS patients

The GRSs were tested for associations with outcome in age- and sex-adjusted and in multivariable-adjusted Cox models. Both GRS32 and GRS47 were significantly associated with recurrent ACS in age- and sex-adjusted models (HR 1.210, 95% CI 1.059-1.382,  $P=0.005$ ; HR 1.199, 95% CI 1.046-1.374,  $P=0.009$ , respectively) and in multivariable-adjusted models (HR 1.165, 95% CI 1.008-1.346,  $P=0.038$ ; HR 1.169, 95% CI 1.009-1.355,  $P=0.037$ , respectively). Figure 7 shows the multivariable-adjusted hazard curves for GRS47, divided into two groups determined by median value. GRS32 was also associated with the composite event of recurrent ACS or CAD death in the age- and sex-adjusted model, but not in the multivariable-adjusted model (HR 1.121, 95% CI 1.121-1.239,  $P=0.026$ ; HR 1.093, 95% CI 0.982-1.122,  $P=0.102$ , respectively). GRS153 had no association with either end-point.



**Figure 7** *Multivariable-adjusted hazard curves for recurrent ACS. Separate lines for patients with low GRS (below the median) and high GRS (above the median). Adjustments for age, gender, hypertension, dyslipidemia, diabetes, prior MI, smoking, subtype of ACS, 3-vessel disease, and pulmonary congestion. ACS indicates acute coronary syndrome; GRS, genetic risk score.*

After confirming GRS32 and GRS47 to be associated with recurrent ACS independent of clinical risk factors, estimation of the clinical value of GRSs was based on ROC curves (II). A clinical risk estimation model was formed by combining age, sex, hypertension, dyslipidemia, diabetes, prior myocardial infarction, smoking, subtype of myocardial infarction, 3-vessel disease, and pulmonary congestion in Cox regression. The AUC for this clinical model was 0.690. The GRSs were then added to this regression model of clinical factors. The AUC for the clinical model supplemented by GRS32 was 0.694. When the clinical model was supplemented by GRS47, the AUC was 0.696. Thus, the addition of either GRS to the clinical model did not improve the AUC clinically significantly.

### 5.2.5. GRS associations with subtype of ACS and clinical factors

All of the GRS correlated inversely with STEMI in age- and sex-adjusted logistic regression analysis (OR 0.380, 95% CI 0.171-0.843,  $P=0.017$  for GRS32; OR 0.357, 95% CI 0.133-0.959,  $P=0.041$  for GRS47; and OR 0.089, 95% CI 0.015-0.523,  $P=0.007$  for GRS153) (II). The unadjusted mean values of the GRSs were only marginally higher in NSTEMI than in STEMI patients (for GRS32, 1.09 vs. 1.08,  $P=0.114$ , for GRS47 1.11 vs. 1.10,  $P=0.259$ , and for GRS153 1.10 vs. 1.09,  $P=0.140$ ) (S.V. et al., unpublished results).

Both GRS32 and GRS47 were, in addition, inversely associated with smoking (OR 0.340, 95% CI 0.148-0.779, P=0.011 for GRS32; P=0.004, OR 0.217, 95% CI 0.077-0.616 for GRS47), and GRS153 with dyslipidemia (OR 0.210, 95% CI 1.375-53.95, P=0.021). GRS32 showed a positive correlation with 3-vessel disease (OR 2.574, 95% CI 1.124-5.896, P=0.025) (II).

### 5.3. Differences between STEMI and NSTEMI patients (III)

#### 5.3.1. Clinical characteristics

The STEMI and NSTEMI patients differed in the prevalence of all risk factors, comorbidities and their CAD severity (Table 8) (S.V. et al., unpublished results). The STEMI patients were younger, and more likely to be men, and to be smokers than were the NSTEMI patients. Hypertension, dyslipidemia, diabetes, and comorbidities were all less common in STEMI patients. Of note, the prevalence of 3-vessel disease was almost twice as high in NSTEMI patients as in STEMI patients.

**Table 8.** *Characteristics and comparison of STEMI and NSTEMI patients in the Corogene cohort.*

	NSTEMI	STEMI	P
N	1134	729	
Age	67.5 ± 11.5	63.3 ± 12.0	<0.001
BMI	27.6 ± 5.0	27.3 ± 4.6	0.176
Heart rate	67.8 ± 15.3	73.1 ± 18.1	<0.001
Male gender	776 (68.4)	532 (73.0)	0.036
Hypertension	797 (70.3)	415 (57.1)	<0.001
Dyslipidemia	852 (75.1)	439 (60.3)	<0.001
Diabetes	258 (22.8)	89 (12.2)	<0.001
Smoking	685 (60.9)	487 (68.0)	0.002
Arrhythmia	223(19.8)	104 (14.4)	0.003
Prior MI	271 (24.1)	105 (14.5)	<0.001
Claudication	130 (11.6)	45 (6.2)	<0.001
Stroke/TIA	160 (14.3)	59 (8.1)	<0.001
3-vessel disease	468 (41.3)	157 (21.5)	<0.001

*Number (percentage within each genotype) is shown for categorical variables. Mean ± standard deviation is shown for continuous variables. NSTEMI, non-ST elevation myocardial infarction; STEMI, ST-elevation myocardial infarction; BMI, body mass index; MI, myocardial infarction; TIA, transient ischemic attack.*

### 5.3.2. Genetic variants associated with NSTEMI in GWAS

Genetic information was available in the Corogene Study for 1 579 MI cases. Most patients (962, 60.9%) had NSTEMI, and 614 (38.9%) had STEMI. At the time of the analyses, only 3 patients with MI were of unspecified ST-segment status.

In GWAS, the threshold for statistical significance was pre-specified at  $P < 5.0 \times 10^{-8}$ . In the analysis of the Corogene Study, two directly genotyped and 14 imputed variants on chromosome 1p13.3 showed a genome-wide significant association with NSTEMI. Of the directly genotyped variants, rs656843 was the most significant (OR for the minor allele 1.63, 95% CI 1.38-1.93,  $P = 1.22 \times 10^{-8}$ ) (Table 9). Of the imputed variants, rs2764553 achieved the highest level of statistical significance (OR for the minor allele 1.62, 95% CI 1.24-1.63,  $P = 1.63 \times 10^{-9}$ ). Conversely, no variant showed any significant association with STEMI or with both MI subtypes combined.

The directly genotyped variant rs656843 was chosen for replication in the TACOS sample. Here, rs656843 showed an association with NSTEMI (OR for the minor allele 1.44, 95% CI 1.12 to 1.86,  $P = 0.005$ ). Moreover, rs656843 was associated statistically significantly with MIs combined (OR 1.38, 95% CI 1.10 to 1.74,  $P = 0.006$ ). No association between rs656843 and STEMI was observable (OR 1.27, 95% CI 0.91–1.77,  $P = 0.16$ ).

When the Corogene and TACOS case-control samples were combined for further analysis, again, rs656843 was associated with NSTEMI, but not with STEMI or MIs combined.

In the FINRISK sample of prospective design, rs656843 showed no significant association with NSTEMI, STEMI, or with STEMI and NSTEMI combined. The estimated HR for NSTEMI was in a similar direction of association (HR 1.13, 95% CI 0.84–1.51,  $P = 0.43$ ), however.

Variants associated with NSTEMI, including also rs656843, are located in the LD block that contains the genes damage-regulated autophagy modulator 2 (*DRAM2*), *CEPT1*, and the 3' end of *DENND2D*. In previous works, rs1335645 between *CEPT1* and *DENND2D* has been associated with plasma gamma-glutamyltransferase (GGT) levels,<sup>31</sup> and rs599839, near *PSRC1* and *SORT1*, has been associated with CAD risk and high LDL levels.<sup>251</sup> No associations appeared between rs656843 and either GGT or LDL in controls of the Corogene sample ( $P = 0.11$  for GGT;  $P = 0.22$  for LDL). Rs656843 was confirmed to be independent from these SNPs by calculation of the correlation between rs656843 and the previously reported SNPs ( $r^2 < 0.05$ ).

When tested in healthy controls, *DRAM2* expression level in blood leukocytes was associated with a majority of the variants near 1p13.3; however, not with rs656843 (expression probe ILMN\_1808634). The most statistically significant variant was rs325927 ( $P = 1.50 \times 10^{-12}$ ).

**Table 9.** *Associations of rs656843 with NSTEMI, STEMI, and MI combined in the study samples.*

Sample	N	OR (95 % CI)	P
Discovery sample: Corogene			
NSTEMI	962	1.63 (1.38-1.93)	1.22E-8
STEMI	614	1.07 (0.88–1.29)	0.50
STEMI, NSTEMI and unspecified MI combined	1 579	1.33 (1.15–1.54)	1.27E-4
Controls	1 576		
Replication I: TACOS			
NSTEMI	389	1.44 (1.12-1.86)	0.0049
STEMI	173	1.27 (0.91-1.77)	0.16
MI combined	562	1.38 (1.10-1.74)	0.0062
Controls	566		
Corogene + TACOS			
NSTEMI	1 351	1.57 (1.36-1.81)	3.11E-10
STEMI	787	1.11 (0.94-1.31)	0.200
STEMI, NSTEMI and unspecified MI combined	2 124	1.34 (1.19-1.52)	2.58E-6
Controls	2 124		
Replication II: FINRISK		HR (95% CI)	
NSTEMI	163	1.13 (0.84–1.51)	0.43
STEMI	99	0.97 (0.65-1.43)	0.87
STEMI, NSTEMI and unspecified MI combined	484	1.00(0.84-1.19)	0.98
Controls	16 143		

*Corogene sample analyzed by logistic regression adjusted for age, sex, and the first ten genomic principal components, and FINRISK by Cox proportional hazards model stratified by study year, geographical region, and genotyping batch; with gender, systolic blood pressure, blood pressure medication, total cholesterol, HDL-cholesterol, smoking and diabetes as covariates. OR indicates odds ratio; CI, confidence interval; HR, hazard ratio; NSTEMI, non-ST elevation myocardial infarction; STEMI, ST-elevation myocardial infarction; MI, myocardial infarction.*

### **5.3.3. Association of previously reported CAD/MI risk loci with NSTEMI and STEMI (III)**

The previously confirmed CAD/MI-related SNPs were tested for association with MI and selectively STEMI or NSTEMI in a case-control setting. In this study, of these 50 variants 16 were associated with MI ( $P < 0.05$ ). Among these were also five SNPs near *CDKN2A/CDKN2B* on chromosome 9p21 (OR for the minor allele of rs10757278 1.28, 95% CI 1.15–1.43,  $P = 1.25 \times 10^{-5}$ ). No statistically significant differences were detectable in MAF between any of the 50 SNPs and STEMI/NSTEMI, when the Bonferroni correction for multiple testing was used (in all tests  $P > 0.001$ ). Interestingly, rs514659 in the *ABO* gene showed the most significant difference (MAF in STEMI patients 48.1%, and 43.3% in NSTEMI patients,  $P = 0.007$ ).

## **5.4. Associations of variant rs656843 at 1p13.3 with cardiovascular risk factors, adverse outcome, and other manifestations of atherosclerosis (IV)**

### **5.4.1. Associations of rs656843 genotypes with clinical characteristics**

The genotype of rs656843 was available for 1 768 patients, of which 56 (3.2%) patients were homozygotes for minor allele C (Table 10). Two-thirds of the patients (1 171, 66.2%) were non-carriers of the risk allele, and 541 (30.6%) were heterozygotes. The MAF of rs656843 was 0.185.

All risk factors and clinical characteristics except ACS subtypes and claudication were evenly distributed across the genotypes. Claudication symptoms were more common in patients with the CC genotype of rs656843 than with CT and TT genotypes (21.4%, 11.2%, and 8.8%,  $P = 0.004$ ) (Table 10). The association of rs656843 with claudication remained significant in an age- and sex-adjusted logistic regression model (OR 1.51, 95% CI 1.15–1.97,  $P = 0.003$ ) and in a multivariable-adjusted logistic regression model (OR 1.48, 95% CI 1.11–1.96,  $P = 0.007$ ): adjustments were for age, gender, hypertension, dyslipidemia, diabetes, smoking, 3-vessel disease, and NSTEMI.

**Table 10.** Clinical characteristics according to rs656843 genotypes and chi-square test results.

	N	Rs656843 genotype			P
		TT	CT	CC	
All patients	1 768	1 171	541	56	
Age	66.8 (57.7-75.2)	66.8 (57.8-75.2)	66.2 (57.0-74.9)	65.8 (57.4-75.8)	0.748
Men	1 225 (69.3)	802 (68.5)	377 (69.7)	46 (82.1)	0.093
NSTEMI	957 (54.1)	597 (51.0)	326 (60.3)	34 (60.7)	0.001
STEMI	616 (34.8)	440 (37.6)	156 (28.8)	20 (35.7)	0.002
3-vessel disease	599 (33.9)	395 (33.7)	183 (33.8)	21 (37.5)	0.844
Hypertension	1 167 (66.0)	770 (65.8)	352 (65.1)	45 (80.4)	0.068
Diabetes	337 (19.1)	225 (19.2)	103 (19.0)	9 (16.1)	0.843
Dyslipidemia	1 253 (70.9)	821 (70.2)	388 (71.7)	44 (78.6)	0.354
Smoking	1 095 (61.9)	728 (62.8)	330 (61.5)	37 (67.3)	0.659
Claudication	174 (9.8)	102 (8.8)	60 (11.2)	12 (21.4)	0.004
Stroke/ TIA	212 (12.0)	144 (12.4)	60 (11.3)	8 (14.3)	0.707

Number (percentage within each genotype) is shown for categorical variables. Median (interquartile range) is shown for age. NSTEMI indicates non-ST-elevation myocardial infarction; STEMI, ST-elevation myocardial infarction; TIA, transient ischemic attack.

#### 5.4.2. Associations of rs656843 with outcome and other manifestations of CVD

A strong association was detectable between rs656843 and PAD: In addition to claudication symptoms, rs656843 (C-allele) was associated with incidence of PAD during the follow-up (age- and sex-adjusted HR 1.66, 95% CI 1.12-2.45,  $P=0.012$  in Cox regression). After adjusting for age, gender, hypertension, dyslipidemia, diabetes, smoking, 3-vessel disease, and NSTEMI, this association remained significant (1.75, 95% CI 1.19-2.59,  $P=0.005$ ) in ACS patients of the Corogene cohort.

Cox regression analysis revealed no association between rs656843 and recurrent ACS, cerebral infarction, or aortic aneurysms. Furthermore, rs656843 was not associated with total, CVD, or CAD mortality.

Because PAD diagnosis was determined by the first PAD-related contact with health care during the follow-up, the diagnosis also reflects the overall burden of PAD symptoms in all study patients. The association of rs656843 with incident and prevalent PAD diagnoses combined was thus calculated. In multivariable-adjusted Cox analysis, rs656843 showed a significant association with PAD in all patients (HR 1.84, 95% 1.40-2.41,  $P=1.061E-5$ ).

The analyses were additionally conducted in the FINRISK population cohort to determine whether the association between rs656843 and PAD was also apparent at population level. Of note, the FINRISK cohort differed from the Corogene cohort

markedly: the individuals were healthier and younger, since the individuals initially included were aged from 25 to 74 years. No correlation between rs656843 and incident PAD was detectable in the FINRISK cohort (HR 1.06, 95% CI 0.89-1.25,  $P=0.522$  in Cox regression adjusted for genotyping cohort and sex; multivariable-adjusted HR 1.03, 95% CI 0.87-1.22,  $P=0.703$ , adjustments for sex, genotyping cohort, cohort year, hypertension, dyslipidemia, and diabetes).



## 6. Discussion

This study evaluated the characteristics of a Finnish cohort of consecutive patients who underwent coronary angiography between 2006 and 2008. GRSs formed of known CAD risk variants were found to be associated with recurrent ACS independent of clinical factors. When compared to other studies evaluating GRS in recurrent ACS prediction, the GRS with 47 confirmed CAD variants included the greatest number of risk variants. Both clinical and genetic differences between STEMI and NSTEMI patients were studied and GWAS analysis revealed variants, including rs656843, at a locus near 1p13.3, to be associated nominally with NSTEMI. Finally, this variant showed a significant association also with PAD in ACS patients.

### 6.1. Cohort evaluation

The Corogene cohort includes comprehensive clinical information on more than 5 000 consecutive patients who underwent coronary angiography. Information from patient records was supplemented with a questionnaire, laboratory tests, and in selected patients, even genotyping. Use of the Hospital Discharge registry and Causes of Death registry from Statistics Finland provided an objective follow-up. In contrast, in many other cohorts, the follow-up is conducted by regular visits or phone calls; thus their follow-up information is more subjective and may be incomplete.

Other cohorts have initially included consecutive patients who underwent coronary angiography, but these cohorts have included only patients with ACS or stable CAD, and excluded patients with stable symptoms and without obstructive CAD in coronary angiography. Thus, only selected parts of the Corogene cohort can be compared to other studies.

For an ACS cohort, the size of the study sample was acceptable. The Corogene cohort is approximately of the same size as other single-center ACS cohorts. However, for example, when compared to the genotyped cohort of the GRACE study,<sup>242</sup> which also tests the association of genetic variants with the outcome of ACS, the Corogene cohort includes 30% fewer patients, and 40% fewer patients with available genetic information. In ACS patients, the proportion of STEMI was similar to that in previous studies, but the distribution to UAP and NSTEMI varies; in this study the proportion of NSTEMI was higher and, accordingly, the prevalence of UAP was lower than in the GRACE study.<sup>41,216</sup>

The mortality rate of ACS patients in the Corogene study was lower (7.5% one-year all-cause mortality) than in some other European cohorts of consecutive ACS patients. For instance, in the GRACE cohort, collected in 2000-2002, the one-year all-cause mortality was almost double (14.8%) that of Corogene,<sup>216</sup> whereas another cohort of NSTEMI patients, collected in the UK in 1998-1999, had an all-cause mortality rate also slightly higher (9.2%) than that of Corogene.<sup>215</sup> Treatment has been rapidly improving, and most likely the timing of the Corogene study accounts for the difference in mortality rates. Evolution in the measurement assays of cardiac biomarkers probably explains the different proportions of the NSTEMI and UAP patients (section 2.2).

It is of interest whether the patients with stable or atypical angina symptoms might benefit from other, non-invasive coronary imaging options or from myocardial perfusion imaging. In the Corogene cohort, one-fourth of the patients needed coronary angiography to rule out CAD as the culprit in their chest pain symptoms and had either no atherosclerotic changes in their coronary arteries or only mild obstruction (<50%) (I). Only one-third of the patients with stable symptoms and obstructive CAD in angiography underwent PCI in the same session or electively later, after the index angiography, and only half of all patients were finally revascularized (I).

Invasive coronary angiography has been the gold standard of coronary imaging for decades.<sup>115,188</sup> It provides a view of the silhouette of the lumen of the coronary arteries while the contrast is administered. By means of invasive coronary angiography without further techniques such as intravascular ultrasound, the structure and pathology of the vessel wall cannot, however, be evaluated. Non-obstructive CAD may also demonstrate vulnerable lesions. They may lie within the vessel wall, and the atherosclerotic coronary arteries may be outward remodeled, so that the lumen diameter can be maintained as normal.<sup>117,228</sup> Outward remodeling cannot be detected by coronary angiography, meaning that a significant atherosclerotic burden and possible vulnerable plaques may be missed. Of note, rupture of a vulnerable plaque may result in thrombosis, and ACS. In theory, diagnostics with coronary angiography alone may thus lead to slightly over-optimistic interpretation. Although among MI patients, revascularization occurs in the same session as the diagnostic coronary angiography, and is the key to ischemia treatment, the role of diagnostic coronary angiography in stable angina patients may be decreasing.

At present, some of the patients included in the Corogene Study could possibly be examined less invasively, for example by CCTA.<sup>72</sup> It provides information on the composition of the plaque.<sup>52</sup> The sensitivity and the specificity have proven good in CCTA; the negative predictive value is high especially in patients with a low pre-test probability for CAD.<sup>91,143,169,185,206</sup> Low-risk patients with stable or atypical chest pain could be diagnosed primarily with CCTA, whereas the invasive imaging method is preferable in patients with a high probability of CAD and always when immediate revascularization is essential.<sup>206</sup> Non-invasive imaging offers benefits when compared to invasive angiography and its ever-present risk for complications. The most common complications in invasive angiography are pseudoaneurysm of the puncture site, and dissection and thrombosis of the vessel.<sup>3</sup> Both methods expose the patient to a risk for contrast reactions. However, the radiation dose is usually higher in CCTA than in invasive angiography,<sup>132</sup> and not all patient groups are suitable for evaluation by CCTA.<sup>206</sup> In evaluation of the hemodynamic significance of a plaque, the invasive angiography supplemented with measurement of fractional flow reserve is superior to CCTA.<sup>140,206</sup> In addition to CCTA, other techniques for advanced imaging of CAD have also been developed, such as myocardial perfusion imaging which shows any ischemia present.

The patients for the Corogene cohort were collected in 2006-2008. Since then, the newer, non-invasive CAD imaging methods have rapidly developed. It remains to be seen to what extent these methods can eventually substitute for the invasive diagnostic angiography.

## 6.2. Genetics affecting outcome of ACS patients

In this study, both an individual SNP and GRSs formed of known CAD risk variants were associated with outcome (II). The risk variant for NSTEMI at 1p13 discovered in this study had no impact on post-ACS prognosis (II-III). Some genetic variants have been associated with the outcome earlier, but the evidence concerning any association between outcome and GRS has been conflicting (sections 2.6.1. and 2.8.2).

Previously, the GRACE Genetics Study showed that known variants at chromosome 9p21<sup>28</sup> and at chromosome 9q34.2 (*ABO* gene region),<sup>242</sup> both of which have been linked to CAD also in the Finnish population,<sup>180</sup> predispose ACS patients to either recurrence or cardiac death during a 6-month follow-up. In the Corogene cohort, however, no correlation between these variants and the outcome emerged (II). Several explanations may exist for this finding. First, the Corogene cohort included substantially fewer genotyped ACS patients. Second, the GRACE Study focused on short-term recurrence and followed the patients for only 6 months, whereas the follow-up of the Corogene cohort was more than 5 years. Third, the effect size of each risk variant may vary across populations. Finally, the proxy SNP for *ABO* in the Corogene and in the GRACE Study differed. In addition to 9p21 and 9q34.2, also loci at 1p13.3 (rs599839) and at 1q41 (rs17465637) can affect the prognosis of patients with prior MI.<sup>49</sup> In the Corogene cohort, these loci showed no association with either recurrent ACS or with the composite end-point of recurrent ACS and CAD death.

The risk variant rs656843 for NSTEMI at 1p13.3, discovered in this study, had no impact on the prognosis after ACS (III-IV). The minor alleles of the variants previously shown to be associated with survival or recurrence are nevertheless considerably more common. Of note, while the MAF of the variant rs1333049 on chromosome 9p21 is 0.48 to 0.53, and the MAF of the variant rs579459, on chromosome 9q34.2 near the *ABO* gene, is 0.21, the MAF of rs656843 is only 0.15. Thus the C-allele of rs656843 is quite uncommon. The potential effect of an allele this uncommon on recurrence/survival may be so modest that it is undetectable in a cohort of only 1768 genotyped patients.

In addition to the 47 confirmed CAD risk SNPs, also over 100 susceptibility loci for CAD were analyzed for this study. In these analyses, locus rs2832227 for the gene *TAK1L*, on chromosome 21, was associated with recurrent ACS and with the composite end-point even after correction for multiple testing and multivariable adjustments for clinical factors (II). This locus is one of the suggestive loci from FDR analysis of the CARDIOGRAMplusC4D study, not reaching the genome-wide significance level. Earlier, this locus rs2832227 has been linked to dilated cardiomyopathy, but that finding could not be replicated.<sup>239</sup> The association between rs2832227 and recurrent ACS, found in the current study, should be further confirmed.

Studies concerning GRS associations with recurrent ACS are emerging. The results have been either negative within one year after ACS,<sup>112</sup> suggestive in a sample with a limited number of events,<sup>231</sup> or positive in patients selected for a randomized controlled trial concerning statin therapy.<sup>139</sup> All of these scores include SNPs that overlap with the SNPs included in the GRSs of this study, but these scores include a maximum 30 CAD risk variants. The GRSs of this study comprise 32, 47, or 153 CAD risk variants and thus cover more of the genetic predisposition to CAD. A different focus with a shorter follow-

up in the Labos study<sup>112</sup> and the limited number of recurrent events in the Tragante study<sup>231</sup> may account for the missing or relatively weak associations. Results from the Corogene study support the finding that GRSs comprising CAD risk SNPs can predict recurrence independently of clinical risk factors, and show this also in a heterogenic population.

Although GRSs have been demonstrated as providing better risk estimations of CAD and first MI than do clinical factors in healthy or at-risk populations,<sup>1,38,83,104,180,221,227,235</sup> here, the addition of the GRS to a clinical risk model did not improve risk prediction for recurrent ACS clinically significantly (II). However, because MI frequency at population level is lower than the ACS recurrence rate, additional variables are necessary to improve the classification.

A GRS that includes only the strongest variants is likely to perform better than a GRS comprising also variants with a weaker effect. In the present study, GRS153 showed no association with either recurrent ACS or with the composite of recurrent ACS and CAD death, although it covers a larger proportion of genetic predisposition to CAD than does either GRS32 or GRS47 (II). GRS153 most likely included some false positives, as it also included variants that were strongly, but not genome-wide significantly, associated with CAD. This weakens its prediction capability. Furthermore, the genetic variants first discovered are the most common and their association with CAD is the strongest.

Bjornsson et al found that a GRS comprising CAD risk SNPs correlates with the presence of multivessel disease.<sup>19</sup> In the Corogene cohort, this finding was confirmed in a Finnish population, as the GRS32 showed an association with 3-vessel disease. In fact, CAD-related SNPs from large-scale GWAS studies are suggested to be linked primarily with atherosclerosis and less with MI.<sup>181</sup> Not only genetic background but also the pathophysiology behind atherosclerosis and MI differs, thrombosis in particular being more important in MI pathophysiology than in CAD. That the only variant related to MI but not to CAD is the *ABO* blood-type gene further supports this theory. Whereas atherosclerosis slowly develops along with multiple risk factors and age, MI occurs in smokers and even in younger individuals with fewer risk factors, indicating a more sudden disease onset.

Of the CAD-related SNPs included in the GRSs in this study, most act either independently of known risk factors, or their action is unknown, but 11 variants act through lipids and five through hypertension.<sup>182</sup> Of note, none of these variants is known to associate with smoking.<sup>259</sup> In this study, the GRSs were inversely associated with smoking, however (II). This finding suggests that to develop ACS, smoking patients may need a somewhat smaller genetic risk burden than do non-smoking patients. This emphasizes the value of smoking as an ACS risk factor.

To conclude, when compared to other studies concerning GRS in recurrent ACS prediction,<sup>112,139,231</sup> the GRS with 47 confirmed CAD variants includes the largest number of risk variants. This GRS was associated with recurrent ACS in a heterogenic Finnish population of ACS patients. The GRS did not, however, improve the accuracy of prognosis prediction clinically significantly. Smoking, showing an inverse association with the GRS, may outweigh the importance of a genetic predisposition to CAD.

### 6.3. Differences between STEMI and NSTEMI patients

Many clinical differences between STEMI and NSTEMI patients are widely acknowledged (section 2.7). The pathology behind these ACS subtypes also differs. In addition to confirming these clinical differences in a Finnish population, this study also revealed the first difference in genetic background between these two ACS subtypes by discovering a genetic variant rs656843 on chromosome 1p13.3 linked exclusively to NSTEMI (III). This finding could also be replicated in another case-control material, but not in the prospective setting. Additionally, in the cohort of ACS patients, the GRSs correlated inversely with STEMI, and thus positively with NSTEMI.

Interestingly, 3-vessel disease is much more common in NSTEMI patients than in those with STEMI, indicating progressive atherosclerosis. Altogether the genetic, pathologic, and clinical differences suggest that the two MI subtypes may have somewhat different background mechanisms, NSTEMI possibly being more of a continuum to the slow process of stable CAD and STEMI arising from local plaques, plaque vulnerability, and thrombosis.

The inability to confirm the association between rs656843 and NSTEMI in the prospective setting possibly results from less uniform diagnostic criteria, inclusion of MIs other than type 1, and a smaller number of cases than in the case-control samples. The case-control replication sample was collected by a single center and contained 389 NSTEMI cases, but the 163 incident NSTEMI cases in the prospective sample were identified from hospital discharge registries.

Of the CAD risk variants found before this study, only one (*ABO*) is a risk variant for MI and not for CAD. As none of the SNPs previously reported to be associated with CAD showed statistically significant differences between STEMI and NSTEMI patients, the genetic variants affecting CAD development predispose individuals similarly both to NSTEMI and to STEMI. Genetic differences between the MI subtypes may be responsible for the acute response to plaque rupture and coronary artery occlusion or to the structure of the plaque itself. Interestingly, in this study, it was the *ABO* gene that most closely approached statistical significance for a difference between STEMI and NSTEMI patients (III).

Among ACS patients, the GRSs showed inverse association with STEMI and thus positive with NSTEMI (II). This association may in part be explained by the fact that CAD variants are related primarily to atherosclerosis. When compared to STEMI patients, patients suffering from NSTEMI are more likely to have multivessel disease.<sup>17</sup> In contrast, smokers tend to develop STEMI rather than NSTEMI.<sup>127</sup>

In the large international GWAS meta-analyses, rs656843 showed no association with risk for CAD/MI.<sup>34,40,198</sup> In those meta-analyses, one of the end-points was MI, i.e. NSTEMI and STEMI combined. Since the sample sizes in those cohorts were considerably larger than that of Corogene, it seems unlikely that phenotypic differences alone would explain why the meta-analyses found no association between rs656843 and CAD/MI. The association discovered in this study is possibly affected by some genetic or environmental factor that is somewhat common in Finland but unusual in other European populations.

To conclude, with both clinical and genetic differences between STEMI and NSTEMI patients were studied, GWAS revealed variants, including rs656843, at a locus near 1p13.3, to be associated nominally with NSTEMI. The GRSs' inverse correlation with STEMI and thus positive correlation with NSTEMI, the finding of a genetic variant linked nominally to NSTEMI, the known differences in pathophysiology of STEMI and NSTEMI, and the fact that patients with NSTEMI and STEMI present with different clinical risk-factor profiles all suggest that these two ACS subtypes may have somewhat different etiology.

#### **6.4. The new risk variant for NSTEMI predisposing to PAD?**

Some of the known CAD risk variants are pleiotropic and affect the development of more than one manifestation of atherosclerosis. Therefore the risk variant rs656843 for NSTEMI (III) was also studied for associations with other manifestations of CVD and cardiovascular risk factors in ACS patients. In this study, the variant rs656843 was, even after multivariable adjustments, associated with incident PAD, but only in ACS patients. These data suggest that the variant rs656843 at 1p13.3, in addition to affecting the development of NSTEMI, may also predispose to PAD (IV).

The genetic contribution to PAD is evaluated as differing from that in CAD or MI development, perhaps with traditional risk factors such as smoking playing an even greater role than in CAD development.<sup>62,70</sup> Still, these two conditions share several genetic variants affecting the development of both conditions.<sup>107</sup> For example, the variant at 9p21, the same common variant that is linked to CAD and affects CAD prognosis, is additionally associated with PAD, abdominal aortic aneurysms, and stroke.<sup>70</sup> This study suggests the variant rs656843 as a pleiotropic variant, affecting development of both NSTEMI and PAD, but this finding should be further confirmed (IV). Moreover, the rs656843 risk allele carriers differed only in terms of PAD incidence, whereas other clinical characteristics and risk factors were distributed evenly across the rs656843 genotypes. Thus, the action of rs656843 may be independent from that of traditional CAD risk factors.

The association between the variant rs656843 and incident PAD was significant only in the ACS patients (IV). In contrast, in a population sample of younger and healthier individuals, no such finding was confirmed. Substantially fewer PAD- than CAD-risk variants have emerged,<sup>62,70,102,107,108,154,224</sup> probably because of both the greater clinical and genetic variance of PAD and the fact that environmental factors may have a stronger effect on PAD than on CAD development.<sup>113</sup> Discovery of new risk variants for PAD will require GWAS of even larger sample sizes. Stratification of patients, for example by diabetes, has been suggested to group similar phenotypes of the disease.<sup>107</sup> The inability to demonstrate any association between rs656843 and PAD in a population cohort, but only in a highly selected ACS cohort, may be the result of PAD's dispersed clinical phenotype and genetics. Another reason for this may be the fact that PAD usually develops in quite old age: some of the carriers of the minor allele in the population cohort will develop PAD later in life.

Most of the SNPs at 1p13.3 associated with NSTEMI in GWAS, but not rs656843, showed an association with the *DRAM2* expression (III). *DRAM2* is suggested to contribute to autophagy induction.<sup>260</sup> Autophagy is a process that affects cellular homeostasis and can promote either cell survival or cell death. For example, in postmitotic cells such as vascular SMCs, endothelial cells, and cardiomyocytes, autophagy mediates survival and function.<sup>131,187,213</sup> In many vascular diseases, including atherosclerosis and restenosis, the process of autophagy is altered.<sup>131</sup> Enhanced and diminished autophagy both are harmful and may contribute to senescence and atherogenesis in vascular SMCs.<sup>63,213</sup> Furthermore, autophagy may also have an impact on sensitivity to ischemia-reperfusion injury in the myocardium.<sup>59</sup> The exact function of *DRAM2* needs further elucidation, however.

*DRAM2* is expressed at least in the heart,<sup>256</sup> in skeletal muscles,<sup>122</sup> and in the tibial artery wall.<sup>122</sup> The Genotype-Tissue Expression (GTEx) database<sup>122</sup> has also reported rs656843 as being associated with expression of *DRAM2*, with the minor allele C reducing its expression. Instead of being causal, the association between rs656843 and *DRAM2* expression may be coincidental.

Patients with PAD more closely resemble patients with NSTEMI than those with STEMI. They are older, have more risk factors, and their symptoms worsen slowly, whereas disease onset in STEMI is usually sudden, and not all STEMI patients have any chest pain symptoms before the actual STEMI. Patients with PAD have atherosclerosis throughout their peripheral vessels, comparable to NSTEMI patients' tendency to have more than one obstructed coronary. The association between genetic variants linked to *DRAM2* and PAD must, however, be confirmed in other cohorts.

## 6.5. Limitations of the study

Some limitations can be found in this study. The Corogene cohort is of Finnish origin, and thus it reflects best the Finnish health care system and treatment systems in addition to patients' clinical and genetic characteristics. Before the results can be generalized, they should be validated in other populations. The size of the cohort and the proportion of the genotyped patients can be viewed critically.

Regardless of inclusion of all the consecutive patients undergoing coronary angiography, these patients are not thoroughly representative sample of all ACS patients. A considerable number of patients are treated conservatively in the same region and thus are not included in the study. Those ACS patients undergoing no angiography are usually older, have more comorbidities, and need more help in everyday life. In particular, they have a poorer prognosis. Thus, the survival rate of the ACS patients in the present study can be considered somewhat over-optimistic. Conversely, this study is a good reflection of those ACS patients evaluated as likely to benefit from invasive treatment.

In the Corogene study, patients with less than 50% stenosis were not considered to have CAD. Although most likely a considerable proportion of the patients truly had no

atherosclerosis in their coronary arteries, the group also included patients with mildly obstructed coronaries. Assuming that a vulnerable plaque can lie inside the vessel wall and obstruct the vessel less than 50% if at all, patients with these kinds of plaques actually still have CAD, although CAD may cause no symptoms before the onset of ACS. The most effective medical therapy for these kinds of patients would be crucial to prevent further obstruction and ACS. Thus, the classification in the Corogene Study may be considered marginally misleading, however, it was as accurate as was considered possible and necessary at the time of the collection of the cohort.

The age-range in the Corogene cohort is broad. The importance of the genetic predisposition to CAD is most likely highest in young ACS patients, so the GRS could serve best for the young. Assessing the multivariate model including GRS separately in patient subgroups according to age was not feasible due to lack of power. Additionally, individuals in the FINRISK study were younger than in the Corogene study. Some of these initially healthy FINRISK-cohort individuals are nevertheless likely to develop MI/PAD later in life.

Although the follow-up of the study was complete, morbidities before study inclusion were recorded with insufficient precision to be informative in the data analyses. Some of the ACS patients had already had an MI before their inclusion in the Corogene study. For such patients, their ACS at study inclusion was actually a recurrent ACS. This fact possibly reduces the strength of the association between the GRS and recurrent ACS. Nevertheless, this was taken into account in the multivariable analysis concerning GRS and recurrent ACS association by adjustment of the analysis for prior MI in Study II. In Study IV, information on confirmed PAD diagnosis before study inclusion was unavailable. Thus, although the self-reported claudication symptoms at study inclusion were noted, such symptoms are not a confirmed sign of PAD. Instead, some of these patients may have another reason than PAD for claudication, leading to errors in the classification into incident or prevalent PAD. Since patients with PAD are generally very likely to have at least one PAD-related contact with health care during any 5 years, the likelihood of false negatives during the 5-year follow-up is, however, minimal.

The GRSs of this study do not include the 10 risk SNPs found by the latest large meta-analysis,<sup>159</sup> nor are these SNPs tested for any association with STEMI/NSTEMI, ACS recurrence, or CAD mortality. Of the studies concerning associations between GRSs and recurrent ACS, however, the GRSs created for the Corogene study include the highest number of established CAD risk variants to date.

## **6.6. Clinical implications and future perspectives**

Although the results of this study lack direct clinical implications, they raise several new questions and possibilities.

ACS treatment involves several drugs. Previously clopidogrel and prasugrel, today most often tikagrelor, serve as standard antiplatelet therapy. Some genetic variants



predispose, however, to incomplete efficacy of these drugs.<sup>202</sup> Additionally, some genetic variants impair the action and activity of statins, which are essential in secondary prevention therapy, and are thus related to increased risk for adverse effects such as myopathy.<sup>79,120</sup> Thus far, testing of pharmacogenetic variants has not been a part of everyday clinical practice. Although the risk score, formed of CAD variants in this study, was associated with recurrent MI, it did not improve the prediction clinically significantly. Instead, a GRS combining both CAD risk variants and pharmacogenetic variants or only pharmacogenetic variants may prove more useful in risk prediction and merits investigation.

Recurrent ACS may result either from occlusion of a stent or from further obstruction of the coronary arteries in sections that are not covered with a stent. Stent obstruction may result from stent thrombosis or from in-stent restenosis.<sup>29</sup> Mechanisms of in-stent restenosis include neoatherosclerosis, prolapse of the disrupted plaque, elastic recoil of the vessel wall, constrictive remodeling, neointimal hyperplasia, or a combination of these processes.<sup>29</sup> Restenosis is reported in 30% of patients with a bare metal stent, while in patients with a drug-eluting stent, restenosis is only half as common.<sup>29</sup> Both family history of CAD and some genetic variants, different from CAD variants, have been discovered to predispose a patient to in-stent restenosis.<sup>161,166,201</sup> Nevertheless, the value of GRSs that include CAD risk variants in predicting in-stent restenosis remains unexplored. Additionally, since autophagy is also known to affect risk for restenosis, the *DRAM2*-related variant rs656843 may in theory predispose to restenosis, as well. Its value in prediction of risk of restenosis needs investigation.

The usefulness of a GRS in prevention of CAD in the Finnish population is currently under study.<sup>58</sup> At the moment, however, the GRSs by themselves or supplemented by clinical factors cannot be utilized in predicting or prevention of ACS recurrence in clinical practice. New CAD- and MI-risk variants are being discovered at an increasing pace, however. As the number of known risk variants increase, the variants should also be included in the CAD risk scores to detect any possible improvement in their prediction capability. Recently, a GRS including only CAD risk variants not acting through any known clinical risk factor proved useful in classification of individuals into low-risk and high-risk categories and targeting the statin therapy.<sup>106</sup> Similarly, exclusion of those risk variants related to risk factors could also strengthen GRS's ability to predict ACS recurrence and improve its prediction capability.

In terms of clinical characteristics, patients with stable CAD resemble NSTEMI patients more than they resemble STEMI patients. Whether the *DRAM2*-related variant rs656843 is additionally related to stable CAD is of interest. Furthermore, confirming an association between ACS and a GRS in patients with stable CAD would help to target prevention even more accurately.

Although *DRAM2* expression has been shown in the left ventricle of the heart, in the tibial artery, and in skeletal muscle,<sup>122</sup> its expression needs further characterization. *DRAM2* expression could be measured in atherosclerotic plaque from a coronary or peripheral artery or from myocardial cells, to further confirm the association between rs656843 and PAD/NSTEMI and to evaluate its importance in their pathogenesis.

## 7. Conclusions

The following conclusions can be drawn on the basis of these studies:

1. This study evaluated the characteristics of a Finnish cohort of consecutive patients who underwent coronary angiography between 2006 and 2008. About half the patients were revascularized. It remains to be seen to what extent new techniques of CAD imaging can eventually substitute for the invasive diagnostic angiography, and whether the proportion of patients revascularized after invasive angiography will rise.
2. The GRS with 47 confirmed CAD variants formed in this study includes the largest number of risk variants among studies concerning GRS in recurrent ACS prediction. This GRS was associated with recurrent ACS in a Finnish population of ACS patients. The GRS failed, however, to significantly improve accuracy of prediction by clinical factors alone. Smoking, with its inverse association with the GRS, may outweigh a genetic predisposition to CAD.
3. Both clinical and genetic differences between STEMI and NSTEMI patients were studied, and GWAS analysis revealed variants, including rs656843, at a locus near 1p13.3, to be associated nominally with NSTEMI. The GRSs' inverse correlation with STEMI, the finding of a genetic variant nominally linked to NSTEMI, and the fact that patients with NSTEMI and STEMI present with differing clinical risk-factor profiles suggest that these ACS subtypes may have somewhat different etiology.
4. The genetic variant rs656843 conferring the risk for NSTEMI was also associated with PAD in ACS patients. Heterogeneity in PAD phenotypes and genetics probably explains why this association could not be shown in a general population cohort, but only in a selected cohort of ACS patients.

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